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NOTES:

In 1937, 64 plants of white clover, *Trifolium repens* L., were chosen from a nursery of approximately 10,000 spaced individuals. These plants varied in such characters as spread, height, density, size of parts, and profusion of flowering. Cuttings from these plants were increased vegetatively in greenhouse beds during the following winter and then redivided into two- or three-node slips for rooting in soil in flats. During the first week in May 1938, these rooted slips were transplanted 1 foot apart in each direction in plots measuring 3 X 7 feet. The soil on which these plots were established was not fertilized in any way throughout the experiment, although it had been lightly manured the previous year when used as a hay field.

The plots were arranged in a lattice design with four replications, according to Goulden's (3) field plan, except that at each "variety" location there were two plots, paired at random and planted from the same clone, one to be clipped high (cutting bar of mower set at 2 inches on level surface) and the other to be clipped low (cutting bar of mower set at 1 inch). In the 6-foot cultivated alley in front of each "variety" location, a single slip of the same clone was planted and allowed

<sup>1</sup>Contribution No. 26 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, in cooperation with the northeastern states. Received for publication August 18, 1941.

<sup>2</sup>Associate Agronomist and Director, respectively.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 6.

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# JOURNAL

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No. 1

## THE EVALUATION OF INDIVIDUAL PLANTS OF WHITE CLOVER FOR YIELDING ABILITY IN ASSOCIATION WITH BLUEGRASS<sup>1</sup>

S. S. ATWOOD AND R. J. GARBER<sup>2</sup>

AS pointed out by Myers and Garber (5),<sup>3</sup> the problem of evaluating pasture plants from a breeding standpoint presents several difficulties, principal of which may be the discrepancy between the behavior of spaced plants and the behavior of these same individuals when growing in sod in association with other species. Although several workers with pasture plants have used small clonal rows or beds, either clipped or grazed, to aid in evaluating the selected individuals, no previous attempts have been made, as far as the authors are aware, to ascertain the performance of individual white clover clones in association with bluegrass.

### MATERIALS AND METHODS

In 1937, 64 plants of white clover, *Trifolium repens* L., were chosen from a nursery of approximately 10,000 spaced individuals. These plants varied in such characters as spread, height, density, size of parts, and profusion of flowering. Cuttings from these plants were increased vegetatively in greenhouse beds during the following winter and then redivided into two- or three-node slips for rooting in soil in flats. During the first week in May 1938, these rooted slips were transplanted 1 foot apart in each direction in plots measuring 3 × 7 feet. The soil on which these plots were established was not fertilized in any way throughout the experiment, although it had been lightly manured the previous year when used as a hay field.

The plots were arranged in a lattice design with four replications, according to Goulden's (3) field plan, except that at each "variety" location there were two plots, paired at random and planted from the same clone, one to be clipped high (cutting bar of mower set at 2 inches on level surface) and the other to be clipped low (cutting bar of mower set at 1 inch). In the 6-foot cultivated alley in front of each "variety" location, a single slip of the same clone was planted and allowed

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to spread at will. At the time of transplanting, a heavy seeding of commercial Kentucky bluegrass was sown uniformly on the plots.

At intervals throughout the summer of 1938 the plots were clipped at a height of about  $1\frac{1}{2}$  inches with a sickle bar power mower to keep down weeds and prevent the ripening of clover seed. Several of the clones bore distinct leaf markings, which made identification easy, and in these plots volunteer plants were seldom found.

In 1939, a lawn mower was used to clip the high-cut plots five times and the low-cut plots six times. The lawn mower used for the high-cut plots was equipped with extension rims on the wheels and extensions on the rear roller. All 1939 cuttings were left on the plots except the last two, which were made during July and August, when yield samples were taken. The samples were collected in a grass catcher by pushing the 18-inch mower once through the long dimension of the plot.

In 1940, both high- and low-cut plots were harvested five times, from May through August. Except for the correlations mentioned in the text, total yields in 1940 were used for the analysis. No separation of clover and grass was made since a very uniform stand of grass was obtained the first summer and subsequent differences in amount of grass appeared to be related to the amount of clover. The samples were dried at about  $120^{\circ}$  F, and all weights were recorded in grams.

The analysis of variance used follows the outline of calculations suggested by Cox and Eckhardt (1), and the probabilities of the F values were determined from Snedecor's (6) table.

#### OBSERVATIONS AND RESULTS

By midsummer 1939 the better clover clones and grass had filled in to form a uniform ground cover and the appearance of the association approximated that of a well-managed pasture. At this time marked differences were noted between clones in amount of clover associated with bluegrass (Fig. 1) and these differences persisted throughout 1940. Since all slips were well rooted in the greenhouse and since the conditions for transplanting were favorable, it would appear that all clones had equally good chances of becoming established and that differences between clones were caused primarily by inherent capability or inability of the clover to grow with the grass. The differences obtained may apply, of course, only at the nutrient level at which the plants were grown. The 1939 and 1940 sods appeared similar to those obtained from seeded mixtures with the possible exception that the better plots had more uniform clover than is usually obtained in sods started from seed. By 1940 the clover was almost completely missing from some plots, whereas in others it was producing an abundance of growth in competition with good stands of grass. These differences in amount of clover were clearly reflected in both the color and the quantity of the associated grass.

One of the objectives of this experiment was to note whether the performance of a spaced individual could be used to predict its performance in sod. In general, poor sods were formed by bunchy types which had extremely short internodal growth, by very prostrate plants which appeared to be smothered out by the grass, and by open-growing clones which maintained this habit in sod. Some of the lower-growing clones performed satisfactorily under close clipping but not

under high, whereas some of the higher-growing clones seemed to adapt themselves to both heights of cutting. Profuse flowering did not appear to reduce vegetative growth as much in sod as it did in spaced plants. The better sods were formed by the taller, more spreading, and more densely growing plants. Growth habit of individual spaced plants, however, was not closely correlated with performance in sod. For example, several cases were observed where adjacent spaced plants appeared very similar in morphological type, but one formed good sod and the other poor. It is concluded that the sod-forming ability of a plant can not be predicted from its performance as a spaced individual and that some test of it in sod is probably necessary in a breeding program.

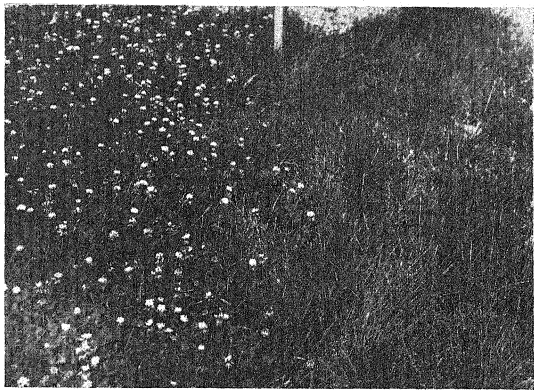


FIG. 1.—Plot at left shows good association of clover and grass; almost no clover has persisted in plot at right.

Differences between clones were noted in their ability to recover after clipping, some growing up much faster than others. It was also noted that, in general, the low-cut plots appeared to recover more slowly after clipping than the high-cut ones.

The yields from the high-cut and low-cut plots were analyzed separately (Tables 1 and 2), since it was seen by inspection of the data that there were differences between them. As suggested by Cox and Eckhardt (1), the lattice experiment was treated as complete randomized blocks for testing the significance of differences among "varieties" or clones. With both the high- and low-cut plots, the  $F$  value exceeded  $P$  of .01, indicating highly significant differences among mean yields of clones. For the high-cut plots, the standard error of the differences between the means of two clones occurring



in the same block is 38.01 and for two clones which do not occur in the same block 39.65. The mean standard error of all comparisons is 39.29. The corresponding values for the low-cut plots are 53.96, 55.87, and 55.45, respectively. With the high-cut plots, the gain in efficiency of the lattice design over the randomized blocks without recovery of inter-block information was 44%, and with recovery of this information 50%. In the case of the low-cut plots, these values were, respectively, 12% and 20%.

TABLE 1.—*Analysis of variance, first, according to lattice design and, second, according to randomized blocks, of total yields in grams during 1940 of 64 high-cut white clover clones grown in association with Kentucky bluegrass.*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Replications.....	3	55,125.07	18,375.02
Component (a).....	14	299,350.78	
Component (b).....	14	154,549.12	
Blocks (elim. clones)...	28	453,899.90	16,210.71
Clones (ign. blocks)....	63	688,703.81	
Error (intra-block).....	161	424,099.28	2,634.16
Total.....	255	1,621,828.06	
Replications.....	3	55,125.07	18,375.02
Clones (ign. blocks)....	63	688,703.81	10,931.81
Error.....	189	877,999.18	4,645.50
Total.....	255	1,621,828.06	

The test of significance of clone differences:  $F = 10,931.81/4,645.50 = 2.35^{**}$ .

TABLE 2.—*Analysis of variance, first, according to lattice design and, second, according to randomized blocks, of total yields in grams during 1940 of 64 low-cut white clover clones grown in association with Kentucky bluegrass.*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Replications.....	3	65,601.38	21,867.13
Component (a).....	14	360,543.99	
Component (b).....	14	161,599.87	
Blocks (elim. clones)...	28	522,143.86	18,648.00
Clones (ign. blocks)....	63	1,068,087.53	
Error (intra-block).....	161	870,082.51	5,404.24
Total.....	255	2,525,915.28	
Replications.....	3	65,601.38	21,867.13
Clones.....	63	1,068,087.53	16,953.77
Error.....	189	1,392,226.37	7,366.28
Total.....	255	2,525,915.28	

The test of significance of clone differences:  $F = 16,953.77/7,366.28 = 2.30^{**}$ .

In Table 3 are shown the frequency distributions for the mean yields of the 64 clones under both high and low clipping. The differ-

ence of 48 grams between the average yield of the high-cut and of the low-cut plots was shown to be highly significant when tested by *t* for paired values, as outlined by Goulden (4). It should be pointed out, however, that 3 of the 64 clones showed a greater mean yield under high clipping than under low clipping. Two of these were relatively low-yielding clones, but one of them was the highest yielding clone among the high-cut plots. This latter plant was an outstanding example of a naturally high-growing clone which appeared capable of adapting its type of growth to either high or low cutting.

TABLE 3.—*Frequency distribution for average yields in grams during 1940 of 64 white clover clones in association with Kentucky bluegrass under high and low clipping.*

Method of cutting	Number of clones yielding following average grams of dry matter																Average yield
	140 to 160	160 to 180	180 to 200	200 to 220	220 to 240	240 to 260	260 to 280	280 to 300	300 to 320	320 to 340	340 to 360	360 to 380	380 to 400	400 to 420	420 to 440		
High cut..	3	2	8	12	11	9	4	8	2	2	—	2	1	—	—	238.9	
Low cut...	—	—	4	6	7	6	6	12	4	3	5	6	3	1	1	286.9	

*t* for low-high = 11.68  
*t* = 2.66 at *P* = 0.01

The distribution of yields throughout the season could not be estimated very well from these data. An attempt was made to clip each time at the same stage of growth, but the early June and the July clippings weighed much more on the average than the other three. It appeared, however, that some of the differences in seasonal yield, such as increased yield in May or August, may have been significant. Considerable growth was made after the August harvest, but no further clippings were made because of threatening frosts in late September.

TABLE 4.—*Correlations calculated from white clover and Kentucky bluegrass yield data obtained in 1939 and 1940.*

Low-cut plots:	
1st cut (May) 1940 with total 1940.....	<i>r</i> = 0.904
2nd cut (June) 1940 with total 1940.....	<i>r</i> = 0.784
3rd cut (June) 1940 with total 1940.....	<i>r</i> = 0.741
4th cut (July) 1940 with total 1940.....	<i>r</i> = 0.878
5th cut (Aug.) 1940 with total 1940.....	<i>r</i> = 0.831
4th + 5th cut 1940 with total 1940.....	<i>r</i> = 0.954
July cut 1939 with total 1940.....	<i>r</i> = 0.719
July + Aug. cut 1939 with July + Aug. 1940.....	<i>r</i> = 0.728
High-cut plots:	
July + Aug. 1940 with total 1940.....	<i>r</i> = 0.950
Low-cut and high-cut plots:	
July + Aug. 1940 (high) with total 1940 (low).....	<i>r</i> = 0.813
Total 1940 (high) with total 1940 (low), using corrected mean values	<i>r</i> = 0.763

Correlations were computed for several combinations of values, as shown in Table 4. According to Fisher's (2) Table VA, all of these values are highly significant. The relationship between these values is high in every case and suggests that possibly one or more clippings could be used in estimating total yields, but of course this would give no information on seasonal distribution of yield. It should be noted that certain of the high correlations may be attributed somewhat to correlating a part of the total yield with the total and in such cases the usual test of significance is not valid. Highly significant correlations were also found between the 1939 and 1940 yields, and between the corrected mean yields of the high-cut and low-cut plots.

### SUMMARY

Sixty-four clones of white clover were increased vegetatively and planted as rooted cuttings in small field plots replicated four times in lattice design. The plots were seeded with Kentucky bluegrass and clipped regularly with a lawn mower. Half of the plots were clipped with the mower set low (1 inch) and half high (2 inches). Yields were taken during the last two clippings of the second summer and throughout the third summer.

Marked differences were noted in ability of the clones to persist in competition with the grass. The presence or absence of good clover was clearly reflected in the color and quantity of the associated grass.

In both the high- and low-cut plots differences between clones in total 1940 yield were highly significant. The lattice design was more efficient than complete randomized blocks.

In general, the taller, more spreading, and more densely growing clones produced better sod, but several exceptions were found.

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## THE EVALUATION OF INDIVIDUAL PLANTS OF PASTURE GRASSES IN ASSOCIATION WITH WHITE CLOVER<sup>1</sup>

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IN any plant breeding program the evaluation of individual plants is of major importance. In pasture and forage crops this problem is particularly difficult since the conditions in the space-planted breeding nursery differ so markedly from those for which the superior strains are being produced. Several workers, including Levy (4),<sup>3</sup> Calder (1), Jenkin (3), and Stapledon (6), have resorted to clonal increases planted in replicated tiller rows as an aid in evaluating individual plants. In addition, Jenkin (3) and Stapledon (6) have used tiller beds in which the clonal pieces were "broadcast" in the evaluation of plants of *Lolium perenne* L., *Dactylis glomerata* L., and other grasses. Stapledon (6) stated that these plantings (tiller rows and tiller beds) were subjected to a system of pasture cuts or to a system of hay and aftermath. Calder (1) grazed the clonal rows with sheep, while Jenkin (3) neither clipped nor grazed the tiller rows in his experiments.

The tiller bed method reported by Jenkin (3) and Stapledon (6) was modified for the present study by the use of a legume (white clover) mixture. The main objective was to determine agronomic variation of the grass clones under the conditions of the experiment.

### MATERIALS AND METHODS

From a space-planted nursery of about 10,000 plants of Kentucky bluegrass, *Poa pratensis* L., 81 plants were selected, representing a wide range of morphological types. During the winter of 1937-38, these plants were increased vegetatively in greenhouse beds, and, in the spring of 1938, they were divided into cuttings of one to three tillers each. The cuttings were rooted in soil in flats and then transplanted to the field in small plots 3 feet by 7 feet in size, the cuttings spaced 7 inches apart each way within the plot. The plots were arranged in a lattice design with four replication. Just before the bluegrass clones were planted, commercial seed of white clover were seeded uniformly over the entire area.

During the summers of 1938 and 1939, the plots were clipped periodically with a sickle bar power mower set at a height of about 1 1/4 inches and the clippings were left on the plots. In 1940, the plots were clipped seven times with a hand-driven lawn mower set at a height of 1 inch and yield samples were taken at each clipping by collecting, in a grass catcher, the clippings from an 18-inch strip through the long axis of the plot. All plots were cut on the same date when the average of the strains was 4 to 5 inches high. The samples were oven dried and all weights were recorded as grams of dry material. Estimations were made several times during the summers of 1938 and 1939 and twice in 1940 of the percentage

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 15.

of ground cover by the grass clone and by white clover on each plot. Since the estimations made at different times agreed well, only those made in June 1940 are used in this report.

For the statistical analysis of the yield data, the arithmetical outline presented by Cox and Eckhardt (2) was followed and the value of  $F$  for  $P$  of .01 was taken from Snedecor (5).

## EXPERIMENTAL RESULTS

### APPEARANCE OF THE PLOTS

By the spring of 1939 most clones had spread sufficiently to fill in between the original cuttings, forming a good sod. With the exception of the plots from four or five clones, the location of each original

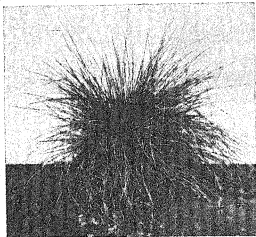


FIG. 1.—Spaced plant of 37-KB140(11) showing extreme bunch habit and narrow leaves.

cutting could be seen at this date, but by midsummer the turf in most plots was so uniform that the original plants no longer were apparent. The plots of three clones were exceptions. One clone had very broad, long leaves and long sheaths and produced few rhizomes, even in the absence of defoliation. In the clipped plots, the plants did not spread and most of them were dead by midsummer in 1939. Another clone was small and weak and, in addition, was so susceptible to *Helminthosporium vagans* Drechsler that it was subject to almost constant defoliation by this patho-

gen. The plants of this clone spread very little during three years and some of them died (Fig. 4, plot on the left). The third clone [KB140 (11)] was an unusual type of Kentucky bluegrass (Fig. 1). Morphologically, it resembled somewhat *Festuca rubra*. The leaves were extremely narrow and tightly folded and the rhizomes were so short that new shoots seemed to arise from the bases of the old. The type of turf formed by this clone (Fig. 2) was sharply contrasted to that formed by the majority of clones (Fig. 3).

Early in the summer of 1938 it became evident that the stand of white clover was not uniform. This lack of uniformity persisted throughout the succeeding two years. There was a great amount of random variation in the stand of white clover. In addition, the differential behavior of the grass clones appeared to be a factor in causing variation. The average percentage of white clover (estimated) for the four replications varied from 6% to 37% among the clones which formed good sods. The average percentages for most of the clones were within the range of 20 to 30%. The plots of two of the weak clones described above had an average of 57 and 60% of clover, suggesting that competition of the grass clone may have been important in reducing the percentage of clover. On the other hand, the plots

of KB140(11) averaged only 22% of clover and this clone could not be considered a strong competitor. The type of sod produced by most of the grass clones and the amount of white clover in most plots (Fig. 4, plot on the right) compared favorably in appearance with the type of Kentucky bluegrass-white clover sod obtained by moderately heavy grazing.

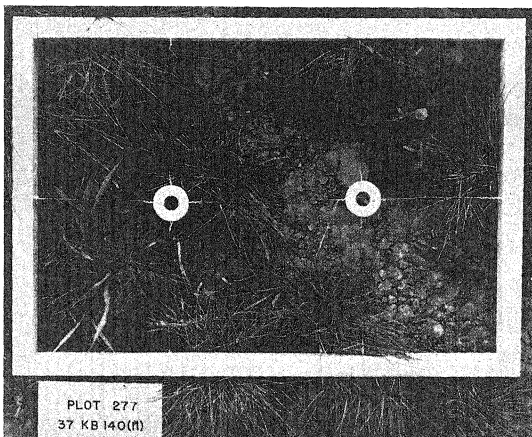


FIG. 2.—Type of sod produced by 37-KB140(11) when cuttings were spaced 7 inches apart in the plot. (Photograph taken May 16, 1939, by V. G. Sprague.)

#### YIELDS OF DRY MATTER

The analysis of variance of the lattice design, in which the variation due to incomplete block was removed, is summarized in Table 1. For the test of significance by means of  $F$  (Table 2), the analysis was converted to that for a randomized block design by combining the "Blocks" component with the "Error". Comparison of mean square for clones with mean square for error exceeded  $F$  for  $P$  of .01, indicating that statistically significant differences occurred among clones in yielding ability. By use of the formulae given by Cox and Eckhardt (2), it was calculated that the standard error of the difference for comparing the means of varieties within the same block was 50.65 grams, while that for comparing the means of varieties in different blocks was 52.60 grams. The standard error of the difference for all comparisons was 52.22 grams.

Using the formula given by Cox and Eckhardt (2) the gain in efficiency of the lattice design as compared with randomized blocks

was found to be 38% without recovery of the inter-block information and 44% with recovery of this information.

TABLE 1.—*Analysis of variance as a lattice design of total yield in grams during 1940 of 81 Kentucky bluegrass clones grown in association with white clover.*

Variation due to	D/F	Sums of squares	Mean square
Rep.....	3	550,272.35	183,424.1166
Component (a).....	16	614,960.3889	
Component (b).....	16	286,469.4567	
Blocks (elim. clones).....	32	901,429.8456	28,169.68267
Clones (ignoring blocks).....	80	837,150.89	10,464.38612
Error (intra-block).....	208	983,614.554	4,728.916125
Total.....	323	3,272,467.64	

TABLE 2.—*Analysis of variance as randomized complete blocks of total yields in grams during 1940 of 81 Kentucky bluegrass clones grown in association with white clover.*

Variation due to	D/F	Sums of squares	Mean square
Rep.....	3	550,272.35	183,424.1166
Clones (ignoring blocks).....	80	837,150.89	10,464.38612
Error.....	240	1,885,044.3996	7,854.351665

F of clone/error = 1.3323; F for P. of .01 = 1.21

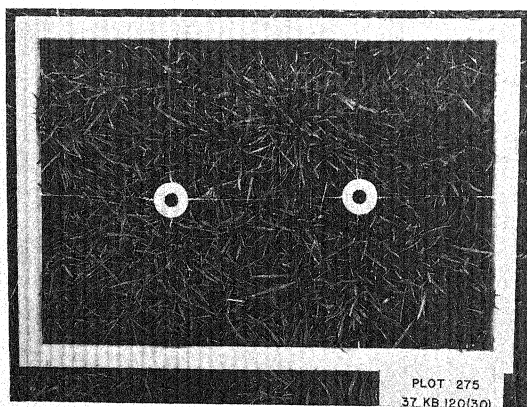


FIG. 3.—Type of sod produced by 37-KB120(30) when cuttings were spaced 7 inches apart in the plot. (Photograph taken May 16, 1939, by V. G. Sprague.)

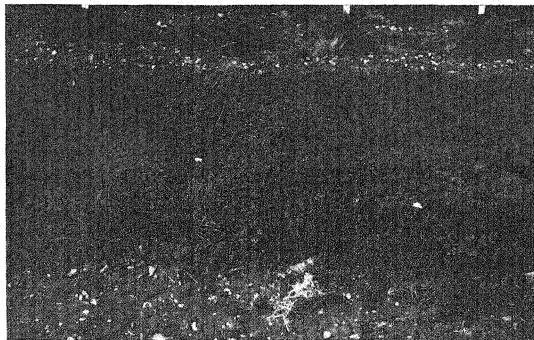


FIG. 4.—Adjacent plots established from different clones of Kentucky bluegrass. The clone in the plot on the left was weak and very susceptible to *Helminthosporium vagans*. The plot on the right shows the grass-clover association typical of most of the clones used in these experiments.

The mean yields of the 20 highest and 10 lowest yielding clones, together with the percentage of bluegrass and white clover in the plots, are given in Table 3. It is worthy of note that the three clones which produced little growth and poor sods are included neither in the lowest 10 nor in the highest 20. In the absence of aggressive bluegrass plants, these plots were occupied with white clover, broad leaf weeds, and other grasses, particularly quack grass (*Agropyron repens* (L.) Beauv.). This mixture outyielded the mixtures of less productive

TABLE 3.—Yield in grams, percentage of planted grass clone, and percentage of white clover of plots of the 20 highest and 10 lowest yielding clones of Kentucky bluegrass.

Clone No.	Yield, grams	Grass, %	Clover, %	Clone No.	Yield, grams	Grass, %	Clover, %
142(223)	570.30	66	29	132(28)	499.50	59	30
130(136)	564.01	71	25	156(85)	496.63	77	17
174(46)	562.06	80	10	144(160)	495.90	62	30
175(46)	551.06	60	29	128(181)	495.72	67	24
176(22)	546.77	72	22	131(6)	492.60	72	21
142(14)	544.21	70	22	96(1)	407.58	70	21
133(211)	543.54	66	32	131(113)	406.07	75	20
135(118)	537.85	68	24	138(599)	403.60	74	22
129(193)	533.85	61	25	139(55)	399.42	79	14
114(12)	520.91	68	25	152(145)	396.81	71	15
52(4)	510.48	56	34	137(69)	396.78	86	6
2(10)	509.67	66	26	120(26)	388.99	66	21
118(4)	505.00	77	17	132(118)	380.89	81	15
144(105)	504.76	49	37	120(30)	358.33	66	26
144(244)	500.52	70	24	1(11)	347.51	76	16



bluegrass clones and white clover but yielded less than the mixtures containing the more productive clones. Another observation drawn from these results was that productivity is not always associated with aggressiveness. KB<sub>120</sub>(30) is an excellent example. This clone was one of the most rapidly spreading clones and the plots of it were among the first in which the original cuttings could no longer be seen. Furthermore, there was an excellent mixture of white clover in these plots (26%). Nevertheless, plots of this clone were next to the lowest in yield among the 81 clones (Table 3).

In consideration of the need for leveling out, in some manner, the low production period in pastures during midsummer, the distribution of yields throughout the growing season is of great importance. When the yields of each clipping for each clone were plotted, the distributions were strikingly similar. The greatest deviations among clones were found in the spring production and two clones were largely responsible for these differences. The yields at different clipping dates of these two clones, KB<sub>174</sub>(46) and KB<sub>175</sub>(46), and of KB<sub>176</sub>(22), the distribution of which was about average, are shown in Fig. 5. The lines connecting yields at different dates of clipping are without significance and were included merely as an aid in following from one clipping to the next of each clone. Among the clones used in this experiment, all showed a similar decline in productivity during the critical midsummer period. Although there were significant differences among the clones in productivity during this period, these differences were associated with the total seasonal yield of the clones.

## DISCUSSION

The method described in this paper was designed for use in preliminary tests of plants to be used in the breeding program. Whether or not the method is suitable for this purpose remains to be determined from the subsequent behavior of the plants tested in this manner. It may be assumed that the most accurate and critical measure of the ability of a plant or strain to persist and produce in pastures will be by use of the grazing animal. On the other hand, tests of large numbers of plants by grazing frequently will be impossible because of the expense. In addition, the use of animals, particularly on small plots, introduces many technical difficulties and sources of error. Therefore, it usually will be necessary to resort to some other method of evaluation in the early stages of the plant breeding program. From a priori reasoning it seems logical to suppose that the most suitable artificial test will be that which most closely approximates the conditions of actual grazing. In several respects the method proposed here meets these conditions. By means of clonal increase the plants can be grown and studied in a sod mixture with white clover. By periodic clipping to stimulate grazing the plants are kept in a vegetative condition and a favorable grass-legume association is maintained. The sod in these plots is so similar in appearance to well-grazed bluegrass that the method may be useful.

Some shortcomings of the method should be noted. These plots were established from clonal pieces and it has not been established

that the behavior of a plant started from a cutting will be the same as that of a plant started from seed. The authors are of the opinion, however, that that usually is not an important factor. The use of clones instead of seed may be of some importance from another standpoint. The behavior of KB140(11) is an example. Such a non-spreading type when compared with spreading types will be at a disadvantage when space-planting of cuttings within the plot is used since such a clone can not occupy the area as fully. On the other

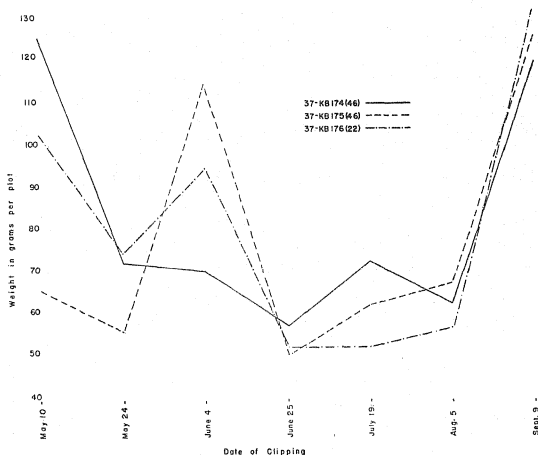


FIG. 5.—Yield in grams per plot on the different dates of clipping during 1940 of three clones of Kentucky bluegrass.

hand, if seed were used, such a type would be expected more nearly to occupy the area merely because the plants would be closer together.

The expense of establishing clonal plots of this type is greater than that of establishing plots from seed, although the certainty of obtaining a good stand is probably greater with the clonal method. In apomictic strains of Kentucky bluegrass, seed could be used as satisfactorily as clones. In cross-pollinated sexual species, however, the use of clones has advantages since seed progenies would not be expected to breed true. Regardless of whether clonal or seed plots are used, it is apparent that the method is too expensive to permit evaluation of all the plants used in a breeding program. It will be necessary to discard as rigidly as possible on the basis of behavior as spaced plants prior to the application of this method. Short

(3-foot) clonal or seeded rows, similar to the tiller rows of Jenkin (3) and Stapledon (6), which can be clipped with a mower are being investigated as an intermediate step between the individual spaced plants and the larger plots. Preliminary observations of these rows are encouraging.

The use of a white clover mixture for evaluating the grass clones has been a much-debated question. In practice, Kentucky bluegrass will be grown in most cases in association with white clover. It seems logical to suppose that the most critical measure of the worth of a grass strain will be obtained by studying it in association with the legume with which it will be used. On the other hand, the presence of white clover in the plots introduces a difficulty in interpreting yield data. Not only does the clover contribute to the total yield, but also the grass clone will be more productive when associated with a legume than when grown in a pure stand. Maintenance of a uniform stand of white clover would eliminate this source of error, but, in practice, a uniform white clover stand is difficult, if not impossible, to maintain. Differential competition from the grass clones or strains is an important factor. The error introduced by differential stands of white clover tends, however, to be overshadowed by certain practical considerations. Since the Kentucky bluegrass strains are being selected for use in association with white clover, the type of mixture obtained with a particular clone is of considerable importance. Thus, a grass clone which is so aggressive that little clover can grow with it will be of questionable value for the production of an improved strain for pasture purposes. In this experiment the average percentage of clover in the plots of most of the grass clones fell within the fairly narrow limits of 20 to 30. Furthermore, the percentage of clover was clearly not the sole factor in conditioning yield differences. For example, the plots of KB174(46), which ranked third in total yield, had an average of only 10% of clover, while, at the other extreme, 5 of the 10 lowest yielding clones had 20% or more clover in their plots.

The usefulness of this method in evaluating plants of bunch type grasses has been investigated on a somewhat more limited scale than with Kentucky bluegrass. Plots have been established of clones of orchard grass, timothy (American or hexaploid), red top, and perennial ryegrass in association with Ladino clover. Results comparable to those reported for Kentucky bluegrass have been obtained for all of these except timothy. Most of the timothy clones did not become established. Since no further attempts to start timothy clones in plots have been made, it is not possible to state at present whether the method is adaptable with this species.

#### SUMMARY

Replicated plots arranged in a lattice design were established from 81 clones of Kentucky bluegrass planted in association with white clover. The plots were clipped periodically to stimulate moderate grazing for three years and the clippings were collected for yield data during the third summer.

A grass-clover association similar in appearance to that found under conditions of moderately heavy grazing was obtained in plots of most of the clones.

Statistically significant differences in total annual yield and differences in seasonal yield were found among the grass clones. The lattice design was more efficient than a randomized block design.

Some of the advantages and disadvantages of the type of test used have been discussed.

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## GRASSHOPPER PROBLEMS ASSOCIATED WITH STRIP CROPPING IN WESTERN KANSAS<sup>1</sup>

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SOIL blowing is one of the more serious agricultural problems of the southern Great Plains. Much progress has been made during the past few years in reducing the soil blowing hazard in western Kansas through the development of a workable, coordinated program in which are incorporated the best known cultural practices for dry land agriculture. While it was assumed that such a program would reduce soil blowing, there was some doubt expressed by agronomists, as well as by entomologists, as to the ultimate success of certain of the practices since they favored the increase of injurious insects, especially grasshoppers.

An opportunity was afforded the authors early in October, 1940, to take data in Greeley County, Kansas, where dry farming practices were in use which featured strip cropping to prevent wind erosion. A widespread grasshopper infestation at wheat seeding time made available a large scale experiment with many variations, each with numerous replications.

### ENVIRONMENTAL FACTORS

Greeley County, located at the center of the west border of the state as shown in Fig. 1 (insert) has had an average rainfall for the past 39-year period of 16.72 inches distributed as shown in Fig. 2. Eleven of the past 15 years have had an annual rainfall below the 16.72-inch average, dropping below 8 inches in 1934.

The crops grown are representative of this part of the southern Great Plains and consist chiefly of winter wheat, spring barley and sorghums.

### STRIP FARMING IN GREELEY COUNTY

Over nine-tenths of the land under cultivation in this county is farmed in long narrow strips. The strips average 10 rods wide and are usually  $\frac{1}{2}$  mile long. Because of the low rainfall of the region, a system of fallowing the crop land during alternate years is generally practiced to reduce the number of crop failures (2).<sup>3</sup> Since the agriculture of the county is primarily devoted to raising wheat, there are many sections where on half of the strips a crop is harvested while the alternate strips are fallowed. Table 1 presents the wheat-fallow rotation as practiced in this county. It may be noted in comparing the wheat-fallow program with the life cycle of the migratory grasshopper, *Melanoplus mexicanus* Saus., that the stubble lands may usually be worked before any of the *mexicanus* eggs laid in the stubble during the previous season have had an opportunity to hatch (Table 1).

<sup>1</sup>This investigation was conducted in connection with Project 211, Bankhead-Jones, and Project 8, Hatch, of the Kansas Agricultural Experiment Station, Manhattan, Kans. Contribution No. 505 from the Department of Entomology, Kansas State College. Received for publication July 15, 1941.

<sup>2</sup>Associate Professor, Assistant Entomologist, and Professor, respectively. The writers wish to acknowledge the assistance of Lee Brewer, former County Agent of Greeley County, for advice and assistance on this problem and of J. E. Taylor and S. Fishman for their aid in acquainting us with the agricultural problems of the county.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 29.



In 1940 there were about 100,000 acres planted to row crops, chiefly sorghums. Some of this was intended to provide grain and forage for livestock, but much of the present acreage was due to the failure of the wheat during the previous winter.

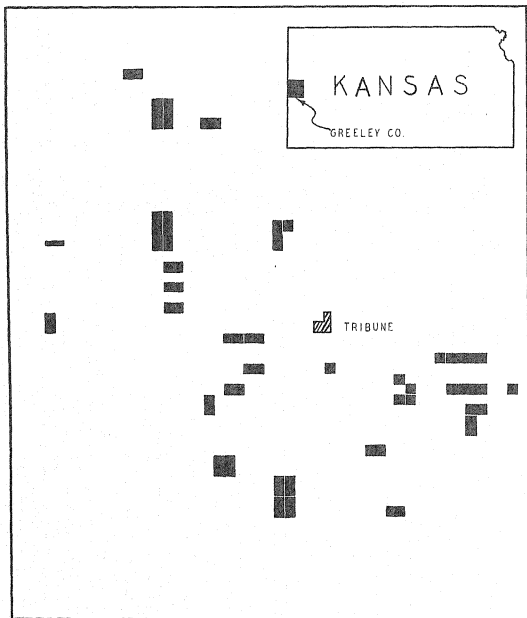


FIG. 1.—Location of Greeley County, Kansas, and distribution of fields surveyed.

#### THE MIGRATORY GRASSHOPPER

Although there have been severe outbreaks of other hoppers, particularly *Melanoplus differentialis* (Thomas) and *M. bivittatus* (Say) in this area, the most abundant and most characteristic hopper infesting the crops of the region is *M. mexicanus*. This hopper is particularly adapted to much of the Great Plains area not only because of favorable temperature and moisture conditions, but also because the general agricultural program favors the oviposition habits of the insect. The favored oviposition sites for the species are stubble lands, weedy idle lands, overgrazed pastures, turnrows, and roadsides.

Every few years the annual cycle of the species in Kansas is characterized by a partial or a complete second generation which hatches during the late summer or fall. Riley (6) noted such a hatch during his observations on the Rocky Mountain locust, though he hesitated to consider it a second generation. During certain years when *mexicanus* eggs hatch in the fall, the nymphs fail to reach the adult stage. In the 1940 season, however, fully 90% became adults. Nearly all of the adult females examined had eggs in the process of development, though comparatively few of these eggs were deposited. During the 1940 season the hatch coincided with the appearance of the newly sprouted wheat and was largely responsible for the damage. The fall grasshopper problem is one which is particularly characteristic of Kansas, eastern Colorado, and adjoining areas to the south. When grasshopper control work is completed over much of the grasshopper infested territory, it frequently happens that a serious problem is just beginning in Kansas.

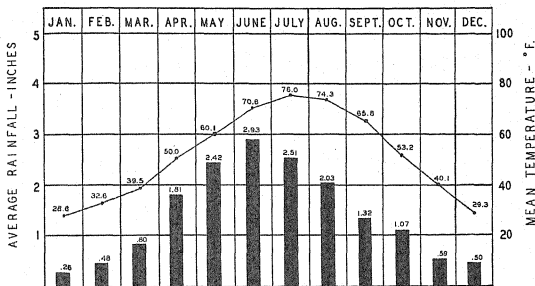


FIG. 2.—Mean temperature and average rainfall for Greeley County, Kans., 39-year average.

#### EXTENT OF FIELD MARGINS WHERE STRIP CROPPING IS PRACTICED

The *mexicanus* problem in fall wheat seeded on fallow land is one of border infestation except where migrating swarms fly into the fields. Grasshopper populations do not develop on fallowed land where the soil has been properly worked. There are two practical aspects of this border infestation; one dealing with the destruction of the wheat from grasshopper feeding and the other dealing with the extent of the area requiring baiting. Assuming that the strips are 10 rods wide (the average width in the Greeley County area) and a mile long, there are 32 strips to the section. In a section of stripped crops, each alternate strip would be in wheat making a total of 320 acres of wheat.

Table 2 shows the extent of borders of 640 acres of wheat planted solid as compared with an equal acreage strip-planted. These figures indicate strikingly the hazards to which the strip-cropped wheat is subjected when the bordering strips are suitable habitats for such injurious hoppers as *M. mexicanus*. A similar situation would be present for other insect pests normally resident in the stubble fields.



TABLE 2.—*Comparison of solid-planted and strip-planted wheat with reference to the extent of field margins, bait requirements, and injury.*

	640 acres wheat solid planted on one section	640 acres wheat alternate strips 10 rods wide, planted on two sections
Miles of wheat field margin.....	4	66
Pounds of wet bait required to bait wheat borders at standard recommendation (80 lbs. wet bait per strip 1 mile long, 2 rods wide).....	320	5,280
Acres of wheat destroyed when hoppers con- sume an average of 1 rod around wheat field margin.....	8	132
Ratio of area of wheat destroyed to area un- damaged.....	1 to 79	1 to 3.8

## EFFECTS OF BORDERING CROPS

A careful survey of representative fields in all parts of the county (Fig. 1) was made to determine under what combination of conditions the most severe hopper injury occurred and the conditions which resulted in comparative freedom

TABLE 3.—*Number of strip margins showing the effects of bordering crops upon the marginal grasshopper feeding.*

Border crops	Amount of injury along margin, feet									Total strip mar- gins
	0	1-3	4-6	7-10	11-15	16-25	26-40	41-60	61+	
Wheat stubble..	7	9	26	15	36	14	21	19	21	168
Barley stubble..	1	11	16	8	8	3	2	0	5	54
Unidentified small grain stubble.....	3	11	1	6	5	6	0	2	6	40
Total for small grain stubble..	11	31	43	29	49	23	23	21	31	262
Milo.....	110	23	17	7	6	3	0	0	0	166
Sorgo (cane)....	19	6	5	2	0	0	0	0	0	32
Kafir.....	7	1	1	0	0	0	0	0	0	9
Mixed sorghums	39	1	1	1	1	0	0	0	0	43
Late sorghums..	18	1	0	1	0	0	0	0	0	20
Sudan grass.....	13	0	3	1	5	1	2	0	0	25
Total for sor- ghums.....	206	32	27	12	12	4	2	0	0	295
Native grass pas- ture.....	1	1	0	0	2	1	0	1	0	6
Abandoned land	1	0	0	1	3	3	1	0	0	9
Roadside.....	5	1	0	2	0	1	0	0	2	11
Total for misc...	7	2	0	3	5	5	1	1	2	26
Corn.....	0	1	1	1	0	0	0	0	0	3

from hopper feeding. A total of 586 field margins were examined, the border crop noted, and the extent of feeding recorded (Table 3). The infestation was somewhat spotted over the county, being considerably greater in the southeastern and northwestern quarters, although all sections suffered to some degree. The extent to which baiting operations were conducted on the various farms influenced the number of hoppers and the extent of hopper injury.

## OBSERVATIONS

### WHEAT BORDERED BY SMALL GRAIN STUBBLE

Small grain stubble, chiefly wheat but including also a considerable acreage of barley, carried large populations of hoppers. Varying amounts of injury were observed, ranging from a few feet of margin to total destruction of the first and second plantings of wheat and, in some cases, partial destruction to even the third planting. Hopper populations in these stubble strips ranged from less than 1 to 75 hoppers per square yard. These variations were in part due to the spotted infestations and in part to the results of the active baiting campaign in progress during the previous month.

Fig. 3 (left) portrays graphically the extent of injury along the margins of wheat fields bordered by wheat and barley stubble. This injury is typically illustrated by Fig. 4. It is not surprising that this injury occurred, considering the extent of the hopper populations in the stubble; in fact, much greater injury might have been expected had it not been for the extensive baiting and the low temperatures during early October.

Given a population of 10 hoppers per square yard, each narrow 20-acre stubble strip bordering the wheat strip carried approximately one million hoppers. While this is not a large hopper population speaking in terms of such outbreaks as have occurred during the past six years in various parts of Kansas, the potential population for the wheat field margins is considerable.

### MOVEMENTS OF HOPPERS TO WHEAT FROM ADJOINING STRIPS

Under certain conditions the hoppers fly from the stubble to the wheat strips, alighting in the center of the field or at any other part. Normally, however, they move to the edge of the field and attack the outer drill row of wheat, followed by the second, third, and so on, until they have moved across the field. In some places, due either to the conditions of the wheat or to a local variation in the hopper population, the advance may be faster than elsewhere giving the field margin an irregular appearance. When the hopper population of a stubble strip 10 rods (165 feet) wide and a mile long is 10 hoppers per square yard, the potential population along each yard of margin on the edge of the wheat is 550 hoppers. If the hoppers in the stubble strip headed for the nearest wheat margin, the population would be 275 per square yard. However, due to prevailing winds or some other factor, there are many fields where the movement tended to be in one direction, as illustrated in Table 4.

As shown in Table 4, there was an average injury of 21 feet along the north margins of the wheat strips as compared with 12 feet of

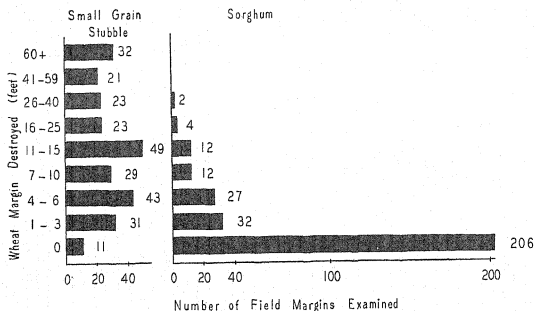


FIG. 3.—Grasshopper injury to wheat bordered by small grain stubble and by sorghum.

injury along the south margin. This is undoubtedly due to a larger number of hoppers having moved out of the Sudan grass in a southerly direction during September. At the time of visitation, however, the greater number of hoppers were moving north since there was an average of 7 hoppers per square yard in the wheat along the southern border of the Sudan grass as compared to 16 in the wheat along the northern border. In most strips examined, with local variations, the movement from the stubble or sorghum strips towards the two bordering strips appeared to be about equal as indicated by number of hoppers and extent of injury.

TABLE 4.—Reversal of movement of hoppers from Sudan grass strips.

Number of wheat strip, Section 1-19-40	Extent of marginal feeding in feet, indicating earlier concentration		Number of hoppers per sq. yd. along margin Oct. 3, 1940, showing a reversal of movement of hoppers	
	North margin	South margin	North margin	South margin
7	37	17	4	11
9	37	17	4	11
11	12	15	10	20
13	10	6	12	25
15	12	6	6	17
Average.....	21	12	7	16

It can be seen that small hopper populations in strips bordering wheat may be significant, for with 1 hopper per square yard in the stubble there is a potential of 55 hoppers per square yard on one of the adjoining wheat field margins. There was some evidence, in addition



FIG. 4.—Strip of fall wheat in Greeley County, Kansas, photographed in early October 1940 showing injury along margins caused by *Melanoplus mexicanus* Saus.

to reports of the farmers, that the hoppers left the barley stubble more promptly than they did the wheat stubble to attack fall-seeded wheat. Practically all volunteer wheat in the wheat stubble had been consumed by October 1, while there were only traces of injury to the volunteer barley in barley stubble.

#### WHEAT BORDERED BY SORGHUMS

As early as 1878, Riley (6) reported that sorghums exhibited a resistance to grasshopper attack. This resistance is more evident in plants over 12 to 15 inches high since younger plants are sometimes destroyed. During the grasshopper outbreaks occurring between 1934 and 1938, the only forage left in many sections of the country were sorghums (1). In Greeley County in 1940 not only were the sorghums relatively uninjured by hoppers, but the wheat adjoining also largely escaped.

Fig. 3 (right) indicates the extent of the injury to the field margins where wheat was bordered by sorghums. An analysis of the percentage of margins showing such injury may be summarized as follows:

	Wheat bordered by sorghums, 295 samples	Wheat bordered by small grain stubble, 261 samples
No injury.....	70%	4%
Slight injury.....	20%	28%
Severe injury.....	10%	68%

In the northwestern part of the county a situation was found where wheat strips on the west side of the county road were bordered by wheat stubble, while those wheat strips east of the road were bordered by a grain sorghum. Fig. 5 presents a portion of this area diagrama-

tically. Where wheat was bordered by stubble, approximately 49 acres were all that remained of the original planting of 160 acres of wheat. The wheat was replanted, where destroyed, only to have 71 acres of the replanted area taken. Thus, counting the two plantings, 182 acres of wheat were destroyed on the original 160 acres sown.

Wheat bordered by stubble - Wheat bordered by sorghum

SECTION 36 16-42

October 5, 1940

SECTION 31 16-41

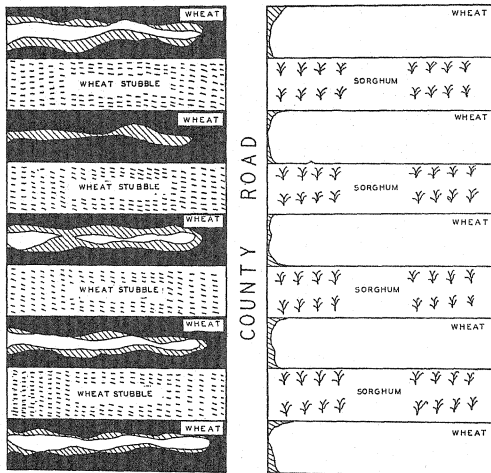


FIG. 5.—Differential injury to wheat by grasshoppers in Greeley County, Kansas.

Across the road, where 160 acres of wheat in strips were bordered by sorghums, a total of 3 acres located along the ends of the strips next to the weedy roadside was destroyed by grasshoppers. The hoppers causing this injury originated in part along the roadside, but many of them migrated across the road from the stubble fields. Table 5 gives the comparison of the grasshopper injury on the two sides of the road.

Further evidence of the border effects of sorghums vs. small grain stubble was available following an examination of eight strips of

TABLE 5.—*Grasshopper injury to wheat bordered by wheat stubble in comparison to wheat bordered by sorghums in adjacent fields of Greeley County, Kansas.*

	160 acres of wheat in strips bordered by wheat stubble, E $\frac{1}{2}$ Sect. 36-16-42	160 acres of wheat in strips bordered by grain sorghum, W $\frac{1}{2}$ Sect. 31-16-41
Average feet margin destroyed, first planting ..	61	4 (along end of strip)
Total acres destroyed, first planting .....	111 (owner actually reseeded 100 acres)	3 (along end of strip)
Average feet of margin destroyed, replanting ..	39	None replanted
Total acres destroyed, replanting .....	71	None replanted
Total acres destroyed, first planting and replanting .....	182	3

wheat located in various parts of the county which were bordered on one side by sorghums and on the other by stubble. The differential border effects are indicated in Table 6.

TABLE 6.—*Differential injury by grasshoppers to eight strips of wheat, 10 rods wide and  $\frac{1}{2}$  mile long, bordered on one side by small grain stubble and on the other by sorghum.\**

	Margin A bordered by small grain stubble	Margin B bordered by sorghum
Average feet of margin destroyed .....	27	4
Total acres of wheat destroyed .....	13	2

\*These strips were located in various parts of the country.

In the majority of sorghum strips, the hopper population was low, though several strips were visited where hopper populations up to 25 per square yard were observed. With the exception of a few fields of Sudan grass which had been sown broadcast for hog pasture, the hoppers present in the sorghum strips had migrated there from nearby small grain stubble or waste lands (probably) after the middle of September. The farmers anticipated injury from hoppers in the sorghums since many of them baited the sorghum strips before seeding wheat. However, the greatest injury to the wheat when bordered by any kind of sorghum occurred where Sudan grass had been cut and shocked. Five strips of wheat bordered by Sudan grass with a hopper population averaging 11 per square yard had border losses of approximately 1 rod.

It was surprising to note that even where a high hopper population was present in sorghums, there was comparatively little feeding either on sorghums or on the adjoining wheat. An example of this is seen in the following data from a field where five strips of wheat were bordered by milo: Number of wheat margins, 10; average extent of border damage, 2 feet; average number of hoppers along margin of

wheat 1 per sq. yd. and in Milo 8 per sq. yd. With 8 hoppers per square yard in the milo strip there was a potential of up to 440 hoppers for each yard along the wheat field margin and yet only 2 feet of the wheat border were destroyed.

To a minor extent the leaves of the sorghums were made ragged by the hoppers and in a few fields the hoppers had gnawed on the grain. From general field observations it is believed that sorghums do not provide a satisfactory food for *M. mexicanus* on which to build up and maintain large populations.

According to Sanderson (7), the replacement of large acreages of cotton with soybeans in Arkansas, beginning in 1933, has resulted in such a large increase in populations of *M. differentialis* that during 1938 it was necessary for 25 counties in the area to conduct grasshopper campaigns. Cotton was unsatisfactory for maintaining populations of the species while soybeans provided the required food elements for rapid growth and high fecundity. According to Painter (4), this has been found to be the case with a number of other insects.

It appears that in western Kansas even a partial replacement of wheat or barley with sorghums would tend to alleviate the *M. mexicanus* problem for the following reasons: (a) The cultivations to eliminate weed growth in sorghum fields during the growing season tends to loosen the soil, thus rendering it undesirable for oviposition by *M. mexicanus*. (b) Sorghums provide a less satisfactory food for growth and fecundity. This point is indicated by field observations but needs to be checked under controlled conditions. (c) Hoppers established in sorghums tend to remain there in spite of an abundance of succulent wheat near at hand. The explanation of this behavior has not been determined.

#### WHEAT BORDERED BY PASTURES, ABANDONED FIELDS, ROADSIDES

There were 26 wheat fields under observation that were bordered by pastures, abandoned fields, and roadsides. As indicated in Table 3 the extent of injury to the wheat in these fields varied from no injury to complete destruction in much the same manner as did that bordered by small grain stubble. The feeding injury at the ends of the entire series of strips corroborated this evidence.

From general field observations it was noted that buffalo grass pastures in good condition carried relatively few *M. mexicanus*. This species was abundant however in weedy pastures and in abandoned lands. These areas invariably contained a heavy covering of Russian thistle, usually two or three species of pigweed, and lamb's quarters. In addition there were annual grasses and varying amounts of the dropseed, *Sporobolus asper*. The abandoned land and weedy pastures carried large populations of *Aeoloplus turnbulli bruneri* Caudell, though by October most of the adults of this species had disappeared.

Throughout western Kansas the roadsides and fence rows play an important part in maintaining high levels of grasshopper populations. In Greeley County the ends of the strips of wheat and the sides of the end strips which came in contact with weedy roadsides invariably showed from moderate to severe damage by hoppers. The extent of the feeding was usually in proportion to the width of the roadside

area and the amount of weeds present. Wherever the wheat field extended up to the wheel tracks of the roadway, as occurred in several instances, practically no injury was present. Fortunately, in Greeley County, there is a minimum of fence rows, fences only being used around pastures. A program is needed for much of the Plains area to reduce the roadside strips and in some sections to eliminate fence rows, for by so doing many of the foci for the infestation of the adjoining land by most hoppers would be eliminated. According to E. G. Kelly, Kansas State College extension entomologist, such a program of roadside elimination is being sponsored with favorable results by county agents in several western Kansas counties.

### THE BAITING CAMPAIGN

The grasshopper situation in western Kansas and neighboring territory is unique and not representative of much of the northern Great Plains region. It is in the fall wheat area and has probably been more frequently subjected to attacks from a second generation of *M. mexicanus* than other regions. As previously suggested, when the hopper problems, including injury, control campaigns, and egg surveys, are over for the year in the northern Great Plains, in western Kansas the hoppers are likely to be starting their most serious damage. The extent to which this was true in Greeley County in 1940 can be seen from the figures reported to the state leader for grasshopper control which show the amounts of wet bait dispensed by the central bait-mixing station at the county seat to be as follows:

Total wet bait mixed in Greeley County up to Aug. 1, 1940, 22 tons; total wet bait mixed in Greeley County beginning Aug. 1, 1940, 674 tons.

One farm operator spread between 25 and 30 tons of bait. Many fields were baited five times or more and the tracks of the trucks and spreaders made regular roadways around the borders of the wheat strips. The reasons for the use of such large quantities of bait and the need for repeated applications may be summarized as follows: (a) The excellent condition of the wheat and the presence of adequate soil moisture gave hopes of a successful harvest if the activities of the hoppers could be stopped. (b) The extent of the field margins under the strip cropping program as indicated in Table 2. (c) The gradual filtering of the hoppers out of the stubble to the wheat margin. This activity began with the first appearance of the young wheat and varying numbers continued migration until a killing frost. (d) The government supplied bait materials. Several farmers admitted that they could not have purchased the required bait materials to carry on the poison campaign as long as had proved necessary.

A check was made on the time of the first applications of bait to protect the young wheat crop and information of a very practical nature was obtained. (Table 7).

When small grain stubble was bordered by wheat, 65% of the margins examined which were baited before seeding fell into the class of slight or no injury, while none were injured severely. When baiting was delayed until after seeding, which meant generally after



damage was observed in the young wheat, only 11% was in the slight or no injury class and 41% rated as severe injury. The differences were not as great where wheat was bordered by sorghums since there were few wheat fields bordered by sorghums that approached the severe injury class; however, the trend was the same.

TABLE 7.—Comparison of grasshopper injury to fall-sown wheat when baited before seeding and when baited after seeding.

Wheat bordered by	Baited	Number of margins examined	Extent of damage (% of margins examined)		
			Slight or no injury (0-3 feet)	Moderate injury (4-15 feet)	Severe injury (16-60 feet)
Small grain stubble	Before seeding	20	65	35	0
	After seeding	90	11	48	41
Sorghums	Before seeding	69	97	3	0
	After seeding	71	80	20	0

#### CULTIVATION METHODS

In Greeley County the eggs of *M. mexicanus* usually hatch in abundance after May 1. From a survey of the cultivation practices used on 33 fields it was found that one farmer made his first cultivation of stubble land in March and the others started cultivation in April, although this work was not completed before June. Parker (5) and Munro (3) have shown that by proper tillage methods before the eggs hatch in the spring, many of the eggs can be destroyed while others can be buried so deeply that the newly emerged nymphs will not reach the surface. These authors agree that the moldboard plow proved the most efficient tool for this work. Munro rated disking second to the moldboard plow, but Parker found this tool to be only half as effective unless equipped with an attachment to aid in turning the furrow slice. Parker considered the duckfoot cultivator as the second best tillage implement for destroying eggs in stubble.

In Greeley County the moldboard plow is seldom used, though a few farmers believe that by using this implement every three years, the "blow-dirt" can more effectively be worked back into the soil. The disk and the one-way, sometimes with a dammer, are used more than any other tools for the original cultivation on stubble lands. It is likely, therefore, that since these tillage implements were less effective than the moldboard plow in the northern Great Plains area, the same thing is true in the Greeley County area and thus many of the eggs deposited on stubble lands escape destruction during the spring cultivation and the young reach the wheat strips.

#### SUMMARY

1. In Greeley County, Kansas, where a large area is devoted to strip farming, certain practices designed to reduce soil blowing have aggravated the injury to fall wheat by *Melanoplus mexicanus*.

2. In this region *M. mexicanus* frequently has a partial or complete second generation which in 1940 coincided with the germination of fall wheat.

3. The extent of the field margins of strip-cropped wheat as compared to solid-planted wheat greatly influenced the amount of feeding injury by *M. mexicanus* as well as the amount of bait required to check the infestation.

4. Strips of fall wheat bordered by small grain stubble suffered much more extensive injury than when bordered by sorghums even though considerable populations of the grasshopper were present in the sorghums, indicating that even a partial replacement of wheat or barley by sorghums tends to alleviate the *M. mexicanus* problem.

5. Weedy pastures, abandoned fields, and roadsides carried populations of *mexicanus* similar to those in the stubble and the injury to adjoining wheat fields was comparable to that of fields adjoining stubble. Improving the condition of the pastures and eliminating the roadside strips are desirable measures for combating the grasshopper problem.

6. Thirty times more bait was used during August and September than was used in May through July.

7. Distributing grasshopper baits before seeding wheat resulted in a marked reduction in the extent of injury to the wheat as compared to baiting only after seeding.

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RELATIVE EFFICIENCY OF INCOMPLETE BLOCK DESIGNS  
USING CORN UNIFORMITY TRIAL DATA<sup>1</sup>M. S. ZUBER<sup>2</sup>

ONE of the salient features of the present-day method of corn improvement consists of the testing of many inbred lines in experimental hybrid combinations. It is not uncommon to include several hundred hybrid strains in trials within a single locality. The soils on which yield trials are planted frequently are quite variable. An experimental design which can effectively cope with the increased soil heterogeneity encountered in large experimental fields is an important asset to enable more accurate selection of the most promising strains. The designs which served satisfactorily for a small number of varieties may be relatively ineffective in eliminating variability due to soil differences in experiments involving a large number of varieties. Sub-dividing the large group of varieties to be tested into several sub-groups and including suitable controls or standard varieties in each sub-group has been used in many trials. Although this method minimizes some of these difficulties, it increases the number of plots of the experiment, thereby adding to the cost of conducting the test. The semi-Latin square design, such as was used in the Iowa Corn Yield Test previous to 1940, enables the testing of a large number of varieties but is subject to a biased error.

For field tests involving a large number of varieties, the incomplete block designs seem to be desirable. These new designs are becoming more general in use and are favorably accepted by those who have used them. Numerous incomplete block designs have been formulated, but four of these designs are at present commonly used, namely, triple lattice, lattice, lattice square, and balanced lattice.

Incomplete block designs employ blocks containing a fewer number of varieties than the total number compared. In the lattice designs the  $k^2$  number of varieties within each replication are grouped into  $k$  blocks of  $k$  varieties each, while in the lattice square designs each replication is arranged as a square of  $k$  rows and  $k$  columns. In the balanced lattice design every pair of varieties appears once in the same block, the number of replications necessary for complete balance being  $k+1$  where  $k$  is an odd number. In the lattice square design where each variety appears with every other variety in either a row or a

<sup>1</sup>Contribution from the Farm Crops Subsection, Iowa Agricultural Experiment Station, Ames, Iowa, cooperating with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture. Jour. paper J-869 of the Iowa Agricultural Experiment Station. Project No. 163. Received for publication July 18, 1941.

<sup>2</sup>Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture; collaborator, Agronomy Section, Farm Crops Subsection, Iowa Agricultural Experiment Station. The writer acknowledges his indebtedness to W. G. Cochran and to Gertrude M. Cox for their valuable suggestions in regard to the statistical methods and to G. F. Sprague and I. J. Johnson for their criticisms and suggestions in preparing this paper. The writer also wishes to thank James Weatherspoon of the Pioneer Hi-Bred Corn Company who so graciously supplied the uniformity data.

column, the number of replications necessary for balance is  $\frac{k+1}{2}$  or a half of the number of replications needed for the balanced lattice.

The lattice and triple lattice designs belong to the unbalanced designs. The variety comparisons in this group are not of equal accuracy because each variety has not appeared in the same number of blocks with every other variety. The number of replications necessary for the lattice design is two, but four replications can be obtained by duplicating the design. The triple lattice requires three replications and similarly provides six replications by duplicating the design.

In practice, the most useful range of number of varieties appears to be from 25 to 160. For 36 and 100 varieties the balanced designs cannot be constructed.

Most corn yield tests in the Corn Belt have plots of about 20 hills in size. In the Iowa Corn Yield Test, a 2 by 12 hill plot has been used for a number of years, while the corn breeding project of the Iowa Agricultural Experiment Station has used a 2 by 10 hill plot.

In the present study, three sizes and shapes of plot were compared, 2 by 10, 4 by 5, and 2 by 5. It was thought that the 4 by 5 hill plot might prove especially suitable with the lattice square design, since it gives a nearly square replication. Also, with the increased number of items to be tested and the nearly equal yielding ability of many of the double crosses in the yield tests of commercial hybrids, it might be desirable to reduce the plot size by one-half and double the number of replications; for example, using a 2 by 5 hill plot with two replications rather than a 4 by 5 hill or a 2 by 10 hill plot with one replication.

The object of this investigation was to determine from a study of corn uniformity data which of the four incomplete block designs and which shape and size of plot would give the highest precision when compared with the randomized complete block.

#### REVIEW OF LITERATURE

Although many studies of field plot technic have been published during the past 25 years, only those investigations which have a direct bearing on this problem will be reviewed. Richey (8)<sup>3</sup> advanced a method for adjusting variety yields based on their regression to a moving average. This method was shown to be effective in eliminating differences due to soil heterogeneity when a large number of varieties were tested.

Yates (10) presented a new method known as the pseudo-factorial arrangement in conducting trials involving a large number of varieties. He found that this arrangement gained 26 to 57% in efficiency over the randomized complete block. In a subsequent study (11) dealing with incomplete randomized blocks, the methods of computation were shown and the relative efficiency of each design was compared. Later, Yates (12) superimposed a quasi-Latin square design of 25 varieties on a uniformity trial with oranges and found a gain in precision of 91% over a randomized complete block.

Goulden (5) studied the results of 26 different comparisons of incomplete blocks and randomized complete block experiments and found the incomplete

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 46.

block method increased the efficiency from 20 to 25%. He also showed that the incomplete block designs gave the greatest gain in efficiency when the blocks were as nearly square as possible.

Weiss and Cox (9) presented the analysis of two quasi-factorial arrangements, the balanced incomplete block and the lattice square, and illustrated their use in soybean trials. They concluded that these designs gave the greatest gain in precision when the number of varieties was large and when the test was located on a heterogeneous soil. The precision gained in one of the lattice square designs was 150% above that of randomized complete blocks, while another lattice square design lost 31.5% in comparison with randomized complete blocks. The former test was conducted on variable soil and the latter on very uniform soil. These computations were made without the recovery of inter-block information.

Yates (13) and Fisher and Yates (3) later proposed a method for the recovery of the information between blocks which had been ignored in the papers cited above. Cox, Eckhardt, and Cochran (4) outlined the new method of computation for the triple lattice and lattice designs with illustrations from data obtained in corn yield trials.

McClelland (7) found that long narrow plots gave a slightly lower error than plots twice as wide and half as long. In uniformity trials with three open-pollinated varieties, Bryan (1) concluded that those varieties were adequately represented by approximately 50 hills of three plants each. In a later study Bryan (2) showed that the variability in plot yields of corn decreased as the size of the plot increased from 8 to 16 and 24 to 48 hills, but the decrease was not proportional to the size of the plot. He also showed that single-row plots had a lower experimental error than a more nearly square plot of the same size. Plot shape was found to be of less importance in affecting experimental error as the size of the plot was reduced.

Immer (6), in a study of size and shape of sugar beet plots, showed that as the size of the plot was decreased and the number of replications increased to utilize the same area of land, the efficiency of the experiment was increased.

## MATERIAL AND METHODS

The data used in this study were obtained by harvesting 3,584 individual plots 2 rows wide by 5 hills long from a field of Pioneer Hybrid 307. This particular field was of a very uniform stand and located on a soil not greatly different in drainage and fertility than that commonly encountered in other corn yield tests. The corn was planted with a horse-drawn, check-row planter at the rate of three kernels per hill. The yield from each 2 by 5 hill plot was adjusted for missing hills according to the following formula:  $CW = FW \frac{H - 0.3M}{H - M}$ , where  $CW$  = corrected

weight,  $FW$  = field weight,  $H$  = size of plot in hills, and  $M$  = number of missing hills. This adjustment for missing hills allows 0.7 for each missing hill and 0.3 to be made up in yield by the surrounding hills.

The adjusted weights from each of the 2 by 5 hill plots were then combined into a 2 by 10 hill plot or a 4 by 5 hill plot to study the effect of plot shape on the efficiency of the various designs.

Each of the four quasi-factorial designs studied, namely, lattice square, balanced lattice, triple lattice, and lattice, was applied to the corn uniformity data. In each of the four designs 25, 49, 81, and 121 varieties were assumed using a 2 by 5, 4 by 5, and 2 by 10 hill plot, thus permitting a comparison of 48 combinations of type of design, number of varieties, and plot size and shape.

The relative value of the designs studied was determined by computing the precision of the design in comparison with that of a randomized complete block. The precision was computed from a comparison of the error mean square (after being multiplied by an efficiency factor) of the design with the error mean square of a randomized complete block. The randomized complete block in each case occupied the same area of land as the design with which it was compared. An example of the method of computation is illustrated below in the comparison of a lattice square design and randomized complete block design, with yields in pounds per plot of 25 assumed varieties of 4 by 5 hill plots in three replications. The computations illustrated are without the recovery of inter block information.

	Replication 1					Row Total
	24.0	25.6	25.1	27.3	28.6	130.6
	24.2	26.7	27.2	30.6	27.7	136.4
	29.1	29.0	28.6	29.9	29.1	145.7
	28.5	28.0	28.7	26.0	29.6	140.8
	27.1	28.2	28.6	29.1	29.0	142.0
Col. Total	132.9	137.5	138.2	142.9	144.0	Replication Total 695.5

	Replication 2					
	26.7	25.8	24.4	21.3	21.7	119.9
	26.2	26.6	27.3	25.1	26.9	132.1
	28.4	27.4	28.3	26.6	28.4	139.1
	26.6	27.5	29.5	27.9	27.5	139.0
	28.3	31.6	27.9	29.7	24.6	142.1
Col. Total	136.2	138.9	137.4	130.6	129.1	Replication Total 672.2

	Replication 3					
	21.9	21.4	22.2	25.2	25.5	116.2
	24.6	25.0	27.7	28.7	27.2	133.2
	28.2	27.5	25.1	28.6	26.2	135.6
	25.5	26.8	28.3	27.6	26.1	134.3
	23.9	22.5	24.1	26.7	25.6	122.8
Col. Total	124.1	123.2	127.4	136.8	130.6	Replication Total 642.1

Grand Total = 695.5 + 672.2 + 642.1 = 2009.8

$$1. \text{ Correction term (C)} = \frac{(2009.8)^2}{75} = 53857.28$$

$$2. \text{ Total sum of squares} = (24.0)^2 + (25.6)^2 + \dots + (25.6)^2 - C = 54219.74 - 53857.28 = 362.46$$

$$3. \text{ Replication sum of squares} = \frac{(695.5)^2 + (672.2)^2 + (642.1)^2}{25} - C = 53914.62 - 53857.28 = 57.34$$

$$4. \text{ Rows and replication sum of squares} = \frac{(130.6)^2 + (136.4)^2 + \dots + (122.8)^2}{5} - C = 54063.09 - 53857.28 = 205.81$$

## 5. Columns and replication sum of squares =

$$\frac{(132.9)^2 + (137.5)^2 + \dots + (130.6)^2}{5} - C = 53970.06 - 53857.28 = 112.78$$

$$\text{Rows} = 205.81 - 57.34 = 148.47$$

$$\text{Columns} = 112.78 - 57.34 = 55.44$$

*Analysis of variance as a Lattice Square of 25 Varieties.*

Source of variation*	D/F	Sum squares	Mean square
Total.....	74	362.46	—
Replications.....	2	57.34	—
Rows.....	12	148.47	12.372
Columns.....	12	55.44	4.620
Error.....	48	101.21	2.108

\*Since the data used are from uniformity trials, variation due to varieties is omitted.

*Analysis of Variance as a Randomized Complete Block of 25 Varieties.*

Source of variation	D/F	Sum squares	Mean square
Total.....	74	362.46	—
Replications.....	2	57.34	—
Error.....	72	305.12	4.237

For the randomized complete block design the error variance of a varietal mean is  $\frac{4.237}{3}$ . In the lattice square design each varietal mean is adjusted for the

fertility levels of the rows and columns in which the variety appears. Because of the adjustment the error variance of a varietal mean cannot be obtained from the error mean square simply by dividing by 3. Yates (11) has shown that the

correct divisor is  $3 \left( \frac{k-1}{k+1} \right)$ , the factor  $\frac{k-1}{k+1}$  being called the efficiency factor of

the design. Thus, to compare the effective error mean squares of the two designs

we may compare  $2.108 \times \frac{k+1}{k-1} = 2.108 \times \frac{6}{4} = 3.162$ . The relative precision is  $\frac{4.237}{3.162}$ ,

or 134%. The above comparison ignores the information contained in the row and column means. If this information is used in calculation of the variety means,

the efficiency factor for a lattice square design  $\frac{k+1}{k-1}$  is replaced by the factor

$1 + \frac{1}{2} (\gamma + \mu)$  where  $\gamma = \frac{(2)(R-E)}{(k-1)(R)}$  and  $\mu = \frac{2(C-E)}{(k-1)(C)}$  and E, R, and C are the error,

row, and column mean squares, respectively. The factors used for the other designs compared in this study will not be given but may be found in the literature.

Example of computation of the factor by the new method of analysis is given below. It must be remembered that this is an example applicable only to uniformity data where actual varieties are omitted.

$$\gamma = \frac{2(12.372 - 2.108)}{(4)(12.372)} = \frac{20.528}{49.488} = .414$$

$$\mu = \frac{2(4.620 - 2.108)}{(4)(4.620)} = \frac{5.024}{18.480} = .271$$

$$1 + \frac{1}{2}(.414 + .271) = 1.342 \text{ new factor}$$

$$(1.342)(2.108) = 2.829$$

$$\frac{4.237}{2.829} = 150\% \text{ gain in efficiency by new method of analysis.}$$

The relation of plot size and shape in each of the four designs was studied by means of the coefficient of variation, so the comparison between plot size and shape in each of the different designs could be made directly. The formula used

for computing the coefficient of variation was  $\frac{s}{m} \times 100$ ,  $s$  being the standard

deviation of a single plot and  $m$  the mean. The standard error of the mean also was computed for each design and plot size and shape. The standard error of the mean was obtained by dividing the standard deviation of single determination

by the square root of the number of replications or  $\frac{s}{\sqrt{n}}$ . In computing the

coefficient of variation and the standard error of the mean the efficiency factor of the particular design was taken into consideration.

## EXPERIMENTAL RESULTS

### COMPARATIVE PRECISION OF RANDOMIZED INCOMPLETE BLOCK DESIGNS COMPUTED WITHOUT THE RECOVERY OF INTER BLOCK INFORMATION WITH RANDOMIZED COMPLETE BLOCK DESIGNS

The precision of the lattice square, balanced lattice, lattice, and triple lattice designs when using a 2 by 5, 4 by 5, and 2 by 10 hill plot is shown in Table 1. The precisions are based on a randomized complete block equal to 100% in efficiency. In this table it is not intended to show the best shape and size of plot, but only to illustrate the relative efficiency of the different designs by means of the precisions. The lattice square design was replicated  $\frac{k+1}{2}$  and  $k+1$  times.

The average gain of all randomized incomplete block designs over the randomized complete block was 25%, or stated in a different way, an incomplete block with four replications was equal in precision to a randomized complete block with five replications. This result was the average of 114 trials. These values of incomplete block designs are in agreement with those demonstrated by Yates (12), Goulden (5), Weiss and Cox (8), and Cox, Eckhardt, and Cochran (4).

By combining tests involving different numbers of varieties and various plot sizes or shapes, the lattice square design gave the greatest

average gain in precision, 32% with  $\frac{k+1}{2}$  replications and 31% with

$k+1$  replications. The balanced lattice, lattice, and triple lattice each gave average gains in precision of 26, 18, and 17, respectively, in comparison with randomized complete blocks. Since the balanced incomplete block design requires more replications than the unbal-



anced incomplete designs, it is not legitimate to compare those designs directly on the basis of relative precision. It seems, therefore, that the most valid comparison would be between designs having an equal number of replications and number of varieties and occupying the same ground. The lattice square of 121 varieties requires six replications for balance, while the triple lattice with the same number of varieties also has six replications. Both designs covered exactly the same field area. The average gain in six trials of the lattice square was 32% and for six trials of the triple lattice 16%, a difference of 16% in favor of the lattice square design.

TABLE 1.—*Relative precision of the lattice square, balanced lattice, lattice, and triple lattice designs without recovery of inter-block information in comparison with randomized blocks as 100 in plots of 2 by 5, 4 by 5, and 2 by 10 hills in size.*

Design	No. of replications	No. of varieties	No. of trials	Relative precision with randomized blocks as 100			
				2 by 5 hill plots	4 by 5 hill plots	2 by 10 hill plots	Average
Lattice square	3	25	18	90	137	167	131
	4	49	6	117	121	149	129
	5	81	6	116	145	151	137
	6	121	6	126	129	141	132
Mean							132
Lattice square	6	25	9	89	132	158	126
	8	49	3	116	120	147	128
	10	81	3	115	145	151	138
	12	121	3	125	129	142	132
Mean							131
Balanced lattice	6	25	9	97	119	177	131
	8	49	3	121	104	159	128
	10	81	3	116	113	149	126
	12	121	3	118	104	134	119
Mean							126
Lattice	4	25	9	84	106	182	124
	4	49	6	111	95	147	120
	4	81	3	100	102	160	121
	4	121	3	115	93	116	108
Mean							118
Triple lattice	6	25	9	93	114	171	126
	6	49	3	104	92	154	116
	6	81	3	87	98	141	109
	6	121	6	116	101	129	116
Mean							117
Av. of all designs							125

Another comparison can be made on the same plot area between the lattice square of 49 varieties with four replications and the lattice also with 49 varieties and four replications. The average gain in precision in six trials for the lattice square and lattice over that for randomized blocks was 29% and 20%, respectively, or a difference of 9% in favor of the lattice square.

The balanced lattice and the lattice square designs with  $k+1$  replications also included the same plots, had the same number of varieties, and each was the average of 18 trials. The lattice square gave an average gain of 31% and the balanced lattice 26% in precision, or a difference of 5% in favor of the lattice square. These results would suggest, therefore, that the lattice square design was somewhat more effective than the triple lattice, lattice, and balanced lattice in reducing experimental error.

#### EFFECT OF THE RECOVERY OF INTER-BLOCK INFORMATION

Table 1 gave the precision of randomized incomplete blocks without the recovery of inter-block information. Table 2 shows the relative precisions for the same designs when inter-block information is recovered. The average relative precision for all tests is 136% as compared with 125% in Table 1. In every case there was a gain in accuracy over the randomized blocks, whereas, in Table 1, 10 cases showed a loss. With the new method of analysis the lattice square designs showed gains of 44 and 43%, respectively, while the lattice designs gained about 33%. These figures show a substantial gain for the new method of analysis over the old method. In computing these figures, the relative weights assigned to the inter- and intra-block estimates were assumed to be correct. Calculations by Yates (13) and Cochran (4) indicate that the error from this source is negligible, except perhaps for the 5 by 5 designs in which the figures given in Table 2 are probably about 3% too high.

#### RELATION OF PLOT SIZE AND SHAPE TO COEFFICIENT OF VARIATION

In Table 3 the coefficients of variation per plot are summarized for the various designs with 2 by 5, 4 by 5, and 2 by 10 hill plots. The average coefficient of variation for all designs and plot sizes and shapes is 6.81%. This value is considerably lower than that commonly obtained in corn yield trials and indicates that the extent of variation from plot to plot was small. In field plot tests with corn a coefficient of variation as low as 6.81% would be indicative of a highly accurate experiment.

The 2 by 5 hill plot was computed as being replicated twice as many times as the 4 by 5 hill or 2 by 10 hill plots. For example, a lattice square of 25 varieties with three replications of 4 by 5 or 2 by 10 hill plots would include the same area as six replications of 2 by 5 hill plots.

From a study of the average coefficients of variation of each of the three kinds of plots, regardless of the type of design, the 2 by 5 hill plot had a much lower average coefficient of variation than either the 4 by 5 or 2 by 10 hill plots. The coefficients of variation for the 2 by

TABLE 2.—*Relative precision of the lattice square, balanced lattice, lattice, and triple lattice designs with recovery of inter-block information comparison with randomized blocks as 100 in plots of 2 by 5, 4 by 5, and 2 by 10 hills in size.*

Design	No. of replications	No. of varieties	No. of trials	Relative precision with randomized blocks as 100			
				2 by 5 hill plots	4 by 5 hill plots	2 by 10 hill plots	Average
Lattice square	3	25	18	112	152	195	153
	4	49	6	129	130	165	141
	5	81	6	124	151	161	145
	6	121	6	133	134	147	138
Mean							144
Lattice square	6	25	9	113	146	184	148
	8	49	3	129	130	162	140
	10	81	3	123	152	161	145
	12	121	3	132	134	148	138
Mean							143
Balanced lattice	6	25	9	108	126	181	139
	8	49	3	126	110	162	133
	10	81	3	118	117	152	129
	12	121	3	122	108	136	122
Mean							131
Lattice	4	25	9	105	129	226	153
	4	49	6	120	108	155	128
	4	81	3	129	111	165	135
	4	121	3	121	103	122	115
Mean							133
Triple lattice	6	25	9	107	123	178	136
	6	49	3	121	110	174	135
	6	81	3	131	113	157	134
	6	121	6	120	107	129	119
Mean							131
Av. of all designs							136

10 hill plots were consistently lower than those for the 4 by 5 hill plots. The coefficient of variation in the 4 by 5 hill plots was lowest for the lattice square designs and was highest for the balanced lattice, lattice, and triple lattice, respectively. The standard errors of the means for the various designs also were computed, but since the same conclusions were reached from them, they are not reported.

#### FIELD COMPARISON OF THE 2 BY 5 HILL PLOT WITH THE 4 BY 5 HILL PLOT

From the information gained in this study it was thought that a comparison should be made between the 2 by 5 hill plot and the 4 by

TABLE 3.—*Coefficient of variation per plot for various designs in relation to size and shape of plots.*

Design	No. of replications	No. of varieties	Coefficient of variation		
			2 by 5 hills*	4 by 5 hills	2 by 10 hills
Lattice square	3	25	5.81	6.73	7.01
	4	49	5.56	7.61	6.84
	5	81	5.65	7.65	6.49
	6	121	5.86	7.70	6.76
Mean			5.72	7.42	6.77
Lattice square	6	25	5.91	6.77	7.08
	8	49	5.53	7.64	6.86
	10	81	5.62	7.64	6.49
	12	121	5.86	7.68	6.64
Mean			5.73	7.43	6.76
Balanced lattice	6	25	5.51	7.13	6.56
	8	49	5.45	8.17	6.58
	10	81	5.64	8.70	6.58
	12	121	6.01	8.56	6.88
Mean			5.66	8.14	6.65
Lattice	4	25	5.59	7.61	6.33
	4	49	5.70	8.55	6.85
	4	81	5.94	8.25	6.74
	4	121	6.24	9.01	6.69
Mean			5.86	8.35	6.65
Triple lattice	6	25	5.61	7.27	6.73
	6	49	6.05	8.93	6.76
	6	81	5.95	8.95	7.18
	6	121	6.11	8.68	6.93
Mean			5.93	8.46	6.90
Grand mean			5.77	7.84	6.88

\*Computed as being replicated twice the number of times indicated in the second column.

5 hill plot in an actual field trial. In the 1940 Iowa Corn Yield Test (14) an 11 by 11 lattice square design, testing 121 varieties in six replications with 4 by 5 hill plots, was used in all of the 12 districts. In district 8, in addition to the regular yield test with the 4 by 5 hill plots with six replications, a yield test was included using the 2 by 5 hill plots with 12 replications. Each yield test involved the same 121 varieties. The tests could not be planted on the same farm because of limitations in the amount of land available for the experiment. The 4 by 5 hill yield test was planted on the Walker farm 3 miles from the Agronomy Farm where the 2 by 5 hill yield test was conducted. The soil in both tests was very heterogeneous. The test plot on the Walker farm had a noticeable soil fertility gradient in one direction

and the plot on the Agronomy Farm had a few high lime spots. The coefficient of variation for the two experiments, as shown in Table 4, was 8.04% for the 2 by 5 hill plots and 9.48% for the 4 by 5 hill plots.

The ratio of the coefficient of variation of the 2 by 5 hill plot to the 4 by 5 hill plot for the uniformity data was (5.77 to 7.84) or 1.35. In this experiment the ratio is 8.04 to 9.48 or 1.17. This is a relatively close agreement between the uniformity data and an actual variety experiment, indicating the suitability of the 2 by 5 hill plots over the 4 by 5 hill plots when the former are replicated twice as many times as the latter.

TABLE 4.—Comparative value of lattice square designs of 2 by 5 hill plots replicated  $k+1$  times and 4 by 5 hill plots replicated  $\frac{k+1}{2}$  times from two actual variety yield tests in 1940.

Plot size	Number varieties	No. reps.	Land involved, acres	Standard error mean in pounds	Mean yield, bu.	Bu. needed sig. diff. 5% level	Coefficient of variation, %
2×5	121	12	4.4	1.482	69.89	6.57	8.04
4×5	121	6	4.4	4.251	85.61	9.37	9.48

#### STUDY OF SHAPE OF INCOMPLETE BLOCK

In the balanced lattice, lattice, and triple lattice designs, the plots of an incomplete block may be arranged either in the shape of a square or a rectangle. Fig. 1 shows one replication of a 9 by 9 incomplete block design with 2 by 5 hill, 4 by 5 hill, and 2 by 10 hill plots arranged in the shape of a square and a rectangle. The square incomplete block has nine plots arranged in three rows of three plots each, while the rectangular incomplete block has one row of nine plots. The former arrangement would not be feasible in a lattice square.

In Table 5 a comparison has been made of the precision, coefficient of variation, and the standard error per plot of a 9 by 9 incomplete block with the three plot sizes and shapes, with square and rectangular arrangement. The same data were used in each case.

The average gain in precision for the 2 by 5 hill plot was the same when the plots were arranged either in a square or a rectangle. The 4 by 5 hill plots had a 56% gain in precision when arranged as a square as compared to 4% when arranged as a rectangle, while the 2 by 10 hill plots gained 28% in the square arrangement and 50% in the rectangular arrangement.

Similar results were obtained by comparing the coefficients of variation and the standard errors of the means. These results indicate the importance of the method of grouping plots to form a compact incomplete block.

#### DISCUSSION

Data obtained from a uniformity trial were used in this study to determine the value of various experimental designs with respect to

TABLE 5.—*Precision, coefficients of variation, and standard errors of the mean for 9 by 9 incomplete block design arranged in square and rectangular blocks.*

Design	Shape of block	Size and shape of plot		
		2 by 5 hills	4 by 5 hills	2 by 10 hills
Precision in Per cent				
Balanced lattice.....	Square	133	168	124
Lattice.....	Square	101	145	142
Triple lattice.....	Square	91	154	119
Mean.....		108	156	128
Balanced lattice.....	Rectangular	116	113	149
Lattice.....	Rectangular	100	102	160
Triple lattice.....	Rectangular	87	98	141
Mean.....		101	104	150
Coefficients of Variation				
Balanced lattice.....	Square	5.42	7.16	7.21
Lattice.....	Square	5.89	6.92	7.17
Triple lattice.....	Square	5.83	7.11	7.81
Mean.....		5.71	7.06	7.39
Balanced lattice.....	Rectangular	5.64	8.70	6.58
Lattice.....	Rectangular	5.94	8.25	6.74
Triple lattice.....	Rectangular	5.94	8.95	7.18
Mean.....		5.84	8.63	6.83
Standard Errors of the Mean				
Balanced lattice.....	Square	0.48	0.62	0.65
Triple lattice.....	Square	0.68	0.80	0.91
Lattice.....	Square	0.84	0.98	1.02
Mean.....		0.66	0.80	0.86
Balanced lattice.....	Rectangular	0.54	0.76	0.59
Triple lattice.....	Rectangular	0.68	1.00	0.83
Lattice.....	Rectangular	0.84	1.16	0.96
Mean.....		0.68	0.97	0.79

plot size and shape. Uniformity data are of value in that they facilitate the combination of basic plot units into plots of different sizes and of different shapes. They also permit a comparison of the relative efficiency of different field designs. In general, the results obtained through the use of uniformity data should serve as an indication of the relative performance of the designs in field experiments with the crops from which the data originate. In order for uniformity data to have a valid meaning, planting, culture, and taking of data must be as similar to actual experimental conditions as is possible. The best information would be obtained from the average of data from several uniformity trials obtained in different years. It must be remembered that the data in this study were obtained from a single uniformity trial.

From these studies it was shown that the average gain in precision of the incomplete block designs without the recovery of inter-block

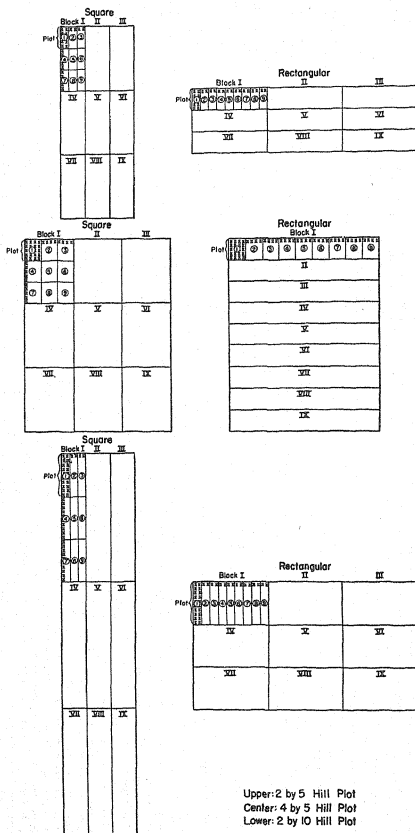


FIG. 1.—Illustrates rectangular and square arrangement of 2 by 5, 4 by 5, and 2 by 10 hill plots in a 9 by 9 incomplete block design.

information was 25% over the randomized complete block. The gain in precision of the incomplete block designs using the inter block information was 36%, an increase of 11% over the earlier method. The uniformity data were obtained from a field of very homogeneous soil as shown by the low coefficient of variation, and one might expect larger gains in precision on a more heterogeneous soil, as is shown in the results obtained by Weiss and Cox (9).

The lattice square designs with both one and two balanced sets of  $\frac{k+1}{2}$  replications were shown to be the most advantageous, followed

closely by the balanced lattice designs. It should be noted that both of these designs belong to the balanced incomplete block group. Theoretically, the lattice square design which removes soil variability in two directions, by rows and columns, should result in greater precision than a design such as a balanced lattice which removed variability in only one direction. With the opportunity to reduce variability by columns, it becomes a question whether the variance thus removed compensates for the accompanying loss in degrees of freedom from the remaining error term. It would seem that in soil of a heterogeneous nature, with no regular gradient and where both rows and columns removed about the same amount of variation, the amount of variance removed by columns would add much to the efficiency of the design. On the other hand, if rows remove five or six times as much variance as columns, showing that the soil heterogeneity is largely in one direction, then the removal of variation by columns may not add greatly to the precision of the experiment. Unless the investigator knows beforehand that such soil variation actually occurs in a known direction, it would seem advisable to use a lattice square design.

The lattice and triple lattice did not give as high relative efficiency in comparison with randomized blocks as the lattice square and balanced lattice designs. Such designs may be applicable, however, in testing a large number of varieties where wide differences may occur, such as in preliminary top crosses and where several tests at different locations are essential, also when the amount of seed and the area of land available are limited.

The fact that the gain in precision did not increase significantly as the number of varieties increased indicates that the soil was of a very homogeneous nature. No losses in precision occurred when the new method of analysis was used. This should add greatly to the attractiveness of incomplete block designs. The randomized complete blocks were apparently as well suited with the larger number of varieties as with the fewer number of varieties. For example, it would be unwise for an investigator to use a randomized complete block for 121 varieties, yet the precision gained by the incomplete block designs was not markedly greater for this number than that gained in the tests of 25 varieties.

From a comparison of the results obtained with the 2 by 5, 4 by 5, and 2 by 10 hill plots with the different designs on uniformity data, it was shown that the coefficient of variation and the standard error of the mean for the 2 by 5 hill plots when replicated twice as



many times were distinctly lower than those from either the 4 by 5 or 2 by 10 hill plots. These results confirm those obtained by Immer (5) in sugar beet plots.

Results obtained from the comparison of the 2 by 5 hill plot with twice as many replications and the 4 by 5 hill plot using a lattice square design in actual yield tests indicated also the 2 by 5 hill plot to be more suitable in reducing the error mean square. However, with a 2 by 5 hill plot size and twice as many replications, twice as many envelopes of seed must be counted out, twice as many tags made, and twice as many plots must be planted, harvested, and weighed. Greater accuracy is necessary in taking plot weights from a 2 by 5 hill plot because of the smaller weights. Smaller harvesting sacks could be used with the 2 by 5 hill plot and the weights were actually taken faster because of the smaller weights and the ease of handling the smaller bags. If a large number of missing hills should occur, the effect on surrounding plots may result in either a large inter-plot error or intra-plot variation.

In considering the use of a 2 by 5 hill plot the effect of varietal competition also must be considered. One of the faults of uniformity trial data is that the results obtained have not been subjected to possible varietal competition. From the standpoint of inter-variety competition the 4 by 5 hill plot should have a distinct advantage for of the 20 hills, 4 have 50% competition, 10 have 25% competition, and 6 are completely surrounded by hills of similar corn. In the 2 by 10 hill plot, 16 hills have 25% and 4 have 50% competition, while in the 2 by 5 hill plot, 6 hills have 25% competition and 4 have 50% competition. It would seem from the standpoint of varietal competition that the 2 by 5 hill plot would be less desirable than a 4 by 5 hill or a 2 by 10 hill plot size.

The lower coefficient of variation and lower standard error of the mean of the 2 by 10 hill plot than the 4 by 5 hill plot in the lattice square is difficult to explain. It has been generally thought that the lattice square design gives the highest efficiency when the plots are square in shape. In a 2 by 10 hill plot the columns of a lattice square design are long and narrow, while the rows have a somewhat more compact shape, depending on the number of varieties in the design. In almost all cases the rows of a 2 by 10 hill lattice square design accounted for nearly two to seven times as much variance as did the columns. The relative efficiency of the 4 by 5 hill plot might have been somewhat higher than the 2 by 10 hill plot if the uniformity trial observations had been made on a more heterogeneous soil. The value of a long, narrow plot has long been recognized as a means of obtaining a lower experimental error than plots of the same size, but more nearly square, especially in randomized complete block designs. The error from the 2 by 10 hill plot was shown to be less than from the 4 by 5 hill plot in the balanced lattice, lattice, and triple lattice designs. In these designs the only variance removed in addition to replications is that accounted for by incomplete blocks. In most cases the 2 by 10 hill plot (depending on the number of varieties in an incomplete block) will give a more compact shaped block than the 4 by 5 hill plot, as shown in Table 6.

TABLE 6.—*Comparative dimensions of incomplete blocks of 5, 7, 9, and 11 plots each with a plot size of 2 by 5, 4 by 5, and 2 by 10 hills.*

Number of varieties	No. plots in incomplete block	Dimensions of incomplete blocks for the plot size indicated		
		2 by 5 hills	4 by 5 hills	2 by 10 hills
25	5	5 by 10 hills	5 by 20 hills	10 by 10 hills
49	7	5 by 14 hills	5 by 28 hills	10 by 14 hills
81	9	5 by 18 hills	5 by 36 hills	10 by 18 hills
121	11	5 by 22 hills	5 by 44 hills	10 by 22 hills

From Table 6, the 2 by 10 hill plot has a more favorable compact shape of incomplete block than either the 2 by 5 or 4 by 5 hill plot. This may account, in part, for the lower error of the 2 by 10 hill plot than the 4 by 5 hill plot. From the standpoint of making field observations, planting and harvesting the 2 by 10 hill plot and the 2 by 5 hill plot would be more advantageous than the 4 by 5 hill plot.

The effect of incomplete block shape in relation to plot size and shape was studied in the 9 by 9 plot arrangement with 81 varieties. With each incomplete block containing nine plots a square incomplete block shaped 3 by 3 plots could be made. In Table 5 it was shown from this study that the square incomplete block with a 2 by 5 hill and 4 by 5 hill plot gained in precision, had a lower coefficient of variation, and lower standard error of the mean than when the incomplete blocks were rectangular in shape. The 2 by 10 hill plots lost in precision and had a larger coefficient of variation and a larger standard error of the mean when arranged in square incomplete blocks.

The dimensions of the incomplete block when the plots are arranged in squares and rectangles for the 2 by 5, 4 by 5, and 2 by 10 hill plots for 81 varieties of nine plots per incomplete block would be as shown in Table 7.

TABLE 7.—*Comparative dimensions of incomplete blocks of nine plots each with a plot size of 2 by 5, 4 by 5, and 2 by 10 hills.*

Shape of incomplete block	Dimensions of incomplete block for the plot size indicated		
	2 by 5 hills	4 by 5 hills	2 by 10 hills
Square arrangement.....	6 by 15 hills	12 by 15 hills	6 by 30 hills
Rectangular arrangement...	5 by 18 hills	5 by 36 hills	10 by 18 hills

The nine plots of 4 by 5 hill size arranged in a square gives an incomplete block shape of 12 by 15 hills, while the 2 by 10 hill plot arranged in a rectangular shape gives an incomplete block 10 by 18 hills. Since the 4 by 5 hill plot is arranged in the most compact shape, it should give the highest accuracy. It was shown that the 4 by 5 hill plot in a square arrangement and the 2 by 10 hill plot in a rectangular

arrangement were essentially equal in precision, coefficient of variation, and standard error of the mean.

The 9 by 9 incomplete block arrangement of 81 varieties is the only one of the four variety combinations studied which permitted an arrangement in square blocks. It would seem that wherever possible in the 9 by 9 incomplete block design the incomplete block should be as compact as possible. This may be done by arrangement of plots of a block in different shapes or by varying the shape of the plot.

### SUMMARY

1. Four incomplete block designs, the lattice square, balanced lattice, lattice, and triple lattice, were applied to corn uniformity trial data assuming 25, 49, 81, and 121 varieties in plots 2 by 5, 4 by 5, and 2 by 10 hills in size. The average of all designs computed without the recovery of inter-block information showed a gain of 25% in favor of the incomplete block designs over the randomized complete block. When these same designs were computed recovering inter-block information an average gain of 36% in favor of the incomplete blocks was shown.
2. Of the various designs studied, computed by the old method, the lattice square with  $\frac{k+1}{2}$  and  $k+1$  replications showed gains of 32 and 31%, respectively. The precision for the other designs was as follows: Balanced lattice, 26%; lattice, 18%; and triple lattice, 17%.
3. The coefficient of variation and standard error of the mean were used to study the adaptation of plot size and shape to the various designs. The results obtained indicated that the 2 by 5 hill plot with twice as many replications yet occupying the same area of land as the 4 by 5 and 2 by 10 hill plots was the most advantageous. The 2 by 10 hill plots and the 4 by 5 hill plots followed in order.
4. The 2 by 10 hill plot was superior to the 4 by 5 hill plot even in the lattice square designs. This was probably due to the fact that the soil heterogeneity ran mainly in one direction.
5. The study of the shape of an incomplete block indicated that the more compact these blocks can be made the more efficient the design will become. The compactness of an incomplete block may be adjusted by the movement of plots within a block or by changing the shape of the plots within an incomplete block.

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RETENTION BY THE SOIL OF THE NITROGEN  
OF SEVERAL AMINO ACIDS<sup>1</sup>JOHN P. CONRAD<sup>2</sup>

THE nitrogen of the various amino acids tested in a previous study (1)<sup>3</sup> showed varying degrees of retention by the soil. The nitrogen of glutamic acid was very weakly retained and that of glycine somewhat more strongly, while that of arginine was very strongly retained. These variations may have originated in different adsorption isotherms for the individual amino acids themselves, or may have arisen from the varying rates at which ammonia or some other strongly retainable nitrogenous compound was split off even during the short period of percolation. In case the amino acids were deaminized the agency might have been either microorganisms, catalysts, or both. The present study was undertaken to investigate and answer these questions.

Since amino acids are the "building stones" of the proteins and, in consequence, are intermediate products in the breakdown of these compounds on the way to form the inorganic ammonium and nitrate forms of nitrogen in the soil, a full knowledge of the processes involved in organic matter decomposition in soils requires a detailed understanding of the interaction of amino acids with the soil. Should the addition of amino acids to soils prove desirable, these results should prove useful in deciding how to add them.

## RETENTION AS INDICATED BY PLANT CULTURES

The retention of the nitrogen of amino acids was determined by the technic of Conrad and Adams (5). By this method 4-inch clay pots previously coated with asphaltum paint were used. With a square of waxed paper over the drainage hole to hold back the dry soil, each pot was charged with 400 grams of Yolo fine sandy loam—a lot deficient in nitrogen under greenhouse conditions. This soil is, in general, neutral to slightly basic containing colloids which are predominantly montmorillonitic and with relatively high cation-exchange capacity. Magnesium is the chief replaceable cation. Four pots were stacked to make a column so that the drainage from one pot dripped into the pot below. Four columns were provided for each treatment employed. The volume of solution for each column was sufficient to wet all of the soil in the column with a slight excess. The solutions for columns receiving nitrogen contained approximately 10 m.at.<sup>4</sup> nitrogen per column and were added in installments to the top pots of the respective columns. The soil designated as "preheated" had been placed in crocks, moistened with distilled water, heated for approximately 48 hours at about 85° C, and then dried. This lot was used to charge all pots in which the soil is designated "preheated".

The columns subjected to percolation for 16 hours received the nutrient solutions in four installments 4 hours apart. For those subjected to percolation for

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 58.

<sup>4</sup>As used in this paper m.at. equals milligram atom.

42 hours, the installments were added about 12 hours apart. Percolation was assumed to be practically finished at the end of 42 hours. After the columns were taken down, the pots were nested in their respective drainage cans and planted to milo. Thirty-six days after planting, the milo was harvested and the final distribution of the nitrogen in the columns was judged by comparisons with the growth in the pots of the distilled water columns.

Table 1 gives the amino acids used, pretreatments, duration of percolation, and the resulting yields of milo. Fig. 1 shows the milo plants in one of the replicates of the glycine cultures.

TABLE 1.—*Growth of milo in pots containing Yolo fine sandy loam variously pretreated and previously stacked and percolated with solutions of different amino acids at different rates of percolation as indicated.*

Percolating solution and soil pretreatment	Duration of percolation, hours	Average yield of green milo, grams per pot			
		1st (top)	2nd	3rd	4th (bottom)
Distilled water:					
None.....	—	2.2	2.3	2.1	2.8
Preheated.....	—	4.5	3.9	4.4	4.3
Glycine:					
None.....	16	16.1	15.4	3.5	2.2
None.....	42	18.4	12.6	3.6	2.0
Preheated.....	16	22.6	19.9	10.8	4.8
Cysteine (HCl):					
None.....	16	20.7	2.5	1.9	1.6
None.....	42	19.0	3.3	1.6	2.2
Preheated.....	42	22.6	7.5	5.7	5.1
Glutamic acid:					
None.....	16	10.9	10.0	9.0	6.3
None.....	42	15.3	9.2	4.9	3.8
Preheated.....	42	18.7	17.5	14.2	7.8

Though differences did occur between the growth in the preheated and untreated soils, the general pattern of the distribution of nitrogen as viewed by crop yields was not markedly changed by variations in the rate of percolation, with the possible exception of glutamic acid, nor by preheating the soil. The generally enhanced growth in the preheated soil may be attributed in part to other factors. The results are very different from those secured with the amides, urea and formamide (4). With these compounds, slowing the rate of percolation decreased significantly the amount of nitrogen getting through to the second pot of the column. The capacity of the soil to retain the nitrogen from percolating solutions of these amides was very greatly reduced by preheating the soil. Subsequent laboratory studies fully confirmed the growth studies as to the retention of nitrogen and indicated that the soils have a weak adsorptive capacity for these and other amides tested and that thermolabile catalysts, enzyme-like in their behavior, existed in these soils and were chiefly responsible for the removal of nitrogen from the percolating solutions.

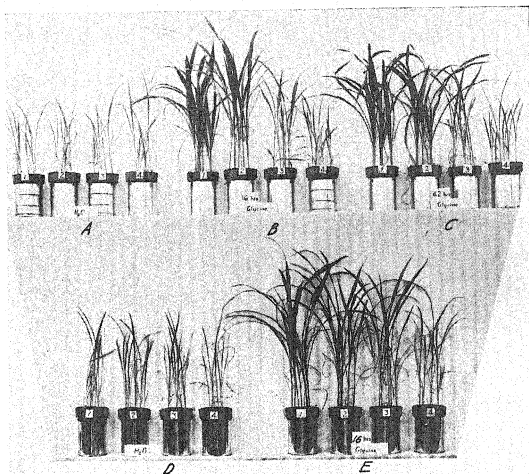


FIG. 1.—Effect of pretreatment and different rates of percolation on the retention of the nitrogen of glycine by dry Yolo fine sandy loam. Each group of four pots marked 1, 2, 3, and 4 had been stacked in a column and subjected to a percolating solution as follows: Columns A and D with distilled water, the others with glycine; B and E in 16 hours, and D in 42 hours. The soil used in columns A, B, and C was untreated, that in columns D and E was preheated.

#### RETENTION AS INDICATED BY ANALYSIS OF SUCCESSIVE PERCOLATES

In studies with urea, formamide, and other amides, tests of the capacity of the soil variously treated to remove the nitrogen of these nutrients from percolating solutions were used to disclose the factors responsible for the transformations. In the present study with amino acids, the soil treatments included normal, i.e., untreated, soil, the same soil treated with toluene, and soil preheated as for the plant culture studies, and then treated with toluene. Presumably, the difference between the first two treatments could be attributed to microbial activity and that between the last two to thermolabile catalysts. In this method, each of several glass percolators provided with a filter disk and filter paper was charged with 1 kilogram of Yolo fine sandy loam with the pretreatments given in Table 2.

In each toluene-treated charge, this antiseptic was mixed intimately with the soil just before it was placed in its percolator. Each solution percolated through toluene-treated soil was itself treated with toluene

in excess of solution saturation. In order to wet all the soil and bring it to the point of dripping, 300 cc of the solution of the given amino acid was added to the soil in the percolator. Thereafter until the end of the test period, 100 cc of the same solution were similarly added every 12 hours. Collection of each percolate from the receiving flask just preceded the addition of a new portion of the percolating solution to the percolator. The concentration of nitrogen in each solution at the start and in each percolate after passing through the soil was determined on suitable aliquots by the usual Kjeldahl method.

The results with the two amino acids, glycine and glutamic acid, are presented in Table 2. After the data from the first percolation experiments were tabulated, it was realized that more than eight percolates should have been collected. For that reason, a second experiment was started as before, but the early percolates were not analyzed. As was to be expected, the results of the second experiments, where they overlapped with the first, showed some differences but these in the main were small.

Percolates from the preheated soil treated with toluene showed small, but positive, reductions in concentration in the later portions. These reductions could be taken as evidence of the presence of weak but definite thermostable catalysts causing the breakdown of the amino acids to form ammonia or some other strongly retained nitrogenous compound. For the most part the reductions with the normal soil—likewise under toluene—were but little greater than those for the preheated soils. The corresponding increases over the preheated soil could most logically be attributed to the activities of thermolabile catalysts. In supplementary incubation studies with each of these amino acids and by the use of the method described by Russell (11, page 567) for ammonia and nitrates, small but approximately equal transformations to ammonia were secured with the two soil treatments in the presence of toluene.

The amounts of nitrogen removed by normal soil without toluene were somewhat greater in the later percolates than by the other treatments. The increases in amounts removed over similar removals with this same soil under toluene could most logically be attributed to microbial activity. The type of behavior, however, is not typical of active multiplication of microorganisms because of the comparative uniformity in the activity displayed. With active multiplication of microorganisms in the presence of abundant energy material, the amino acids, evidence of a rapid increase in the amount of nitrogen removed from solution would have been anticipated. Practically complete recovery of the nitrogen of these two amino acids was secured as ammonia (11) after 8 days of incubation in untreated soil. Though microbial activity seems the safer explanation of these data at this time, the action of thermolabile catalysts—also inactivated by toluene—must not be overlooked.

#### RETENTION AS INDICATED BY ADSORPTION TESTS

In the light of former studies with urea (2, 3, 6) and other amides (4) and the evidence presented herewith, adsorption or some similar phenomenon seems to be the chief factor in the retention of the



TABLE 2.—Reductions in concentrations of the nitrogen of various amino acids in successive 100-cc percolates from 1-kg portions of Yolo fine sandy loam as influenced by the presence of toluene and by the preheating of the soil, values in m. at. nitrogen per liter.

Percolating solution	Glycine				Glutamic acid			
	None	Toluene			None	Toluene		
Antiseptic during percolation	None	None			None	None		
Soil preheating				at 85° C				at 85° C
Experiment No.	1	2	1	2	1	2	1	2
Original concentration	40.0	38.7	40.0	38.7	41.1	39.3	41.1	39.3
Successive 12-hour percolates	Reductions below original concentrations							
1	37.6	—	37.4	—	24.6	—	24.7	—
2	35.6	—	36.4	—	10.6	—	6.6	—
3	29.1	—	29.8	—	6.2	—	4.2	—
4	21.6	—	19.8	—	5.4	—	3.3	—
5	16.0	—	9.5	—	5.8	—	3.4	—
6	13.9	—	5.7	—	7.4	—	3.4	—
7	11.4	8.2	5.1	1.6	11.4	13.6	3.4	2.8
8	11.3	7.9	3.3	1.4	13.6	14.8	2.8	2.6
9	—	7.8	—	1.4	—	14.8	3.4	2.7
10	—	7.3	—	1.2	—	13.2	—	2.6
11	—	7.0	—	1.1	—	12.2	—	2.5
12	—	6.4	—	0.9	—	12.0	—	2.5
13	—	6.4	—	1.0	—	11.0	—	2.5
14	—	7.1	—	0.9	—	10.2	—	2.9
15	—	5.7	—	0.7	—	10.2	—	2.8
16	—	6.2	—	0.8	—	9.6	—	2.4

nitrogen of amino acids by the soil. The growth studies showed that preheating the soil did not materially change the type of behavior as it had with the amides, and the percolation experiments showed large removals of nitrogen in the early percolates, diminishing rapidly with all treatments and nearly to zero with the preheated soil treated with toluene. In adsorption phenomena, a percolating solution would be expected to come to equilibrium with the soil sooner or later and then to percolate through a soil column practically unchanged. For these reasons it seemed desirable to investigate adsorption of these amino acids by the usual laboratory procedures. The smaller quantities involved allowed the inclusion of some amino acids which would have been too expensive to use in the percolation procedures described thus far. For this purpose the following compounds were used and were of the highest quality obtainable from the Eastman Kodak Company:

<i>d</i> Arginine monohydrochloride	<i>d l</i> Alanine
<i>l</i> Histidine monohydrochloride	$\beta$ Alanine
<i>d</i> Lysine monohydrochloride	<i>d l \beta</i> Phenylalanine
$\epsilon$ Amino <i>n</i> caproic acid	<i>l</i> Asparagine
Norleucine ( <i>d l \alpha</i> amino <i>n</i> caproic acid)	<i>l</i> Aspartic acid
Glycine	<i>d</i> Glutamic acid
<i>dl</i> serine	

In addition the following, obtained from the Pfanstiehl Company, were also used: Leucine C.P. and cysteine hydrochloride C.P.

In general, 50 cc of various concentrations of the amino acid in question were shaken in the presence of excess toluene with 100 grams of preheated soil for 2 hours and then filtered. Other soil-water ratios were used with some of the amino acids. The extracts were too highly colored for the direct formol titration method of determining the amount of the amino acid in solution. In consequence, the usual Kjeldahl method was used to determine the water-soluble nitrogen in the filtrates. Small corrections were made on the basis of the water-soluble nitrogen of the soil secured by similar procedures with distilled water.

It is assumed that these corrected figures gave an accurate measure of the amounts of the amino acid remaining in solution. It is believed errors arising from this assumption are small. From these figures were computed the amounts adsorbed by a kilogram of soil in equilibrium with the remaining concentration. In order to avoid undue confusion of lines, the results are reported in two graphs. In Fig. 2 the amounts of adsorption of amino acids are plotted logarithmically against a wide range of equilibrium concentrations. Similarly, in Fig. 3, curves which lie close together are plotted on an enlarged scale. Advantage was taken of this division to facilitate certain comparisons given later.

The indications of the growth and percolation studies are borne out by these adsorption studies. Thus, arginine, which was completely removed by the soil in the top pot of a three-pot column, was adsorbed most strongly by the soil (1). Glycine (Table 1), a small amount of the nitrogen of which penetrated to the bottom pot, was

adsorbed by the soil less strongly. The soil which allowed considerable quantities of nitrogen of glutamic acid to get through to lower pots in the growth studies adsorbed this amino acid only weakly but somewhat more strongly than it did the nitrogen of formamide (4).

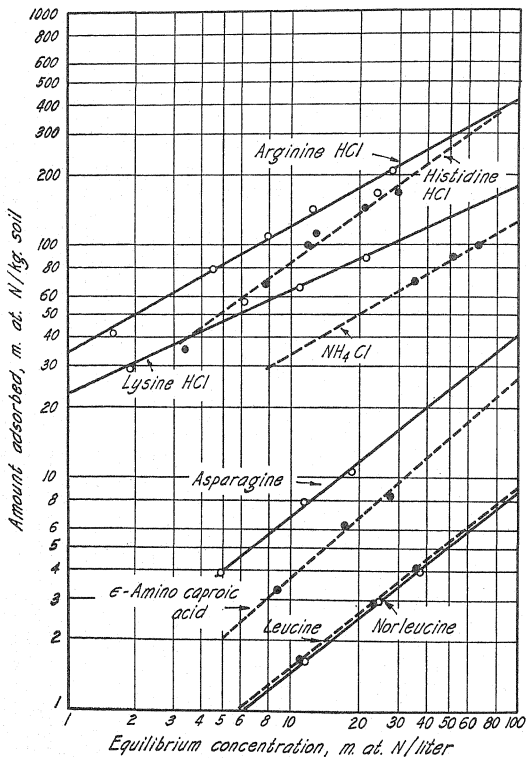


FIG. 2.—Adsorption of some amino acids by preheated Yolo fine sandy loam.

In a former study (1), it was suggested that cysteine may have changed to the much less soluble cystine by chemical oxidation under

the more alkaline conditions of the soil. The observation that much more nitrogen was removed from solution with a 24-hour (Fig. 3) contact of the soil and the cysteine solution, with an apparent increase in adsorption, is in agreement with this explanation. A similar

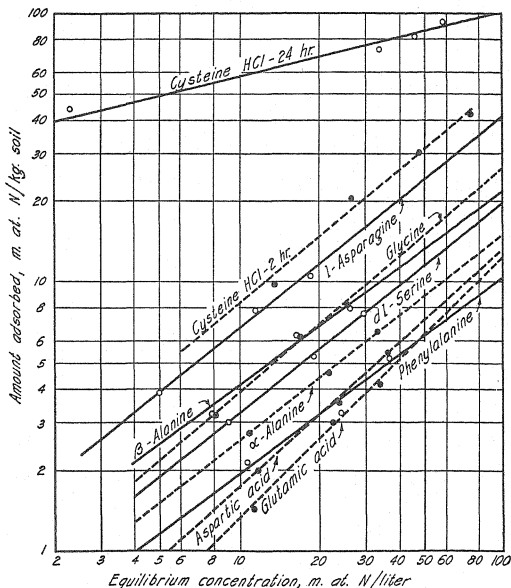


FIG. 3.—Adsorption of additional amino acids by preheated Yolo fine sandy loam.

shaking of glycine solutions with the preheated soil for 24 hours resulted in no material change from the 2-hour results.

The list of some 15 amino acids and  $\text{NH}_4\text{Cl}$  gave an opportunity to draw conclusions with some and to speculate with others on the effect of molecular structure and chemical properties on the intensity with which these compounds were adsorbed by the soil used. For want of evidence it was assumed that *d* and *l* forms were adsorbed equally by the soil. As would be expected the three  $\alpha$ -amino acids with additional  $\text{NH}_2$  or other basic groups were more strongly adsorbed than were the other amino acids. They were more strongly

adsorbed than was the  $\text{NH}_4$  ion. These amino acids have higher pI values, pH of the isoelectric points (9), than do the other amino acids.

Glycine,  $\text{CH}_2\text{NH}_2\text{COOH}$ ;  $\alpha$ -alanine,  $\text{CH}_3\text{CHNH}_2\text{COOH}$ ; and norleucine,  $\text{CH}_3(\text{CH}_2)_3\text{CHNH}_2\text{COOH}$ , all straight-chain amino acids with practically equal isoelectric points, might be expected to be adsorbed with equal intensity, but as the chain was lengthened the intensity of adsorption by the soil diminished. The number of hydrogen-saturated carbon atoms in the chain seemed to be the principal factor involved as leucine,  $(\text{CH}_3)_2\text{CHCH}_2\text{CHNH}_2\text{COOH}$ , with a forked chain, was practically as strongly adsorbed as was norleucine which has a straight chain.

Movement of the amino group ( $-\text{NH}_2$ ) farther away from the carboxyl group ( $-\text{COOH}$ ) resulted in stronger adsorption. Thus,  $\beta$ -alanine,  $\text{CH}_2\text{NH}_2\text{CH}_2\text{COOH}$ , was more strongly adsorbed than  $\alpha$ -alanine,  $\text{CH}_3\text{CHNH}_2\text{COOH}$ .  $\epsilon$ -Amino  $n$  caproic acid,  $\text{CH}_2\text{NH}_2(\text{CH}_2)_5\text{CH}_2\text{COOH}$  was much more strongly held than norleucine, ( $\alpha$  amino- $n$ -caproic acid),  $\text{CH}_3(\text{CH}_2)_5\text{CHNH}_2\text{COOH}$ .

A close examination of Figs. 2 and 3 discloses that the soil had adsorbed almost equally glycine,  $\beta$ -alanine, and  $\epsilon$ -amino- $n$ -caproic acid. Structurally, each of these compounds has its amino group on the end of the carbon chain. In compounds where the amino group is not terminal, the retention of the amino acid molecule by the soil was less. These facts suggest (a) that the adsorption of the amino acid by the soil arises from the attraction between the positively charged amino group of the "zwitterion" (8) and the negatively charged soil particle; (b) that in amino acids where the amino group is terminal, these two opposite charges may more closely approach each other, thereby in accordance with Coulomb's law increasing the attractive force between them; and (c) that where the amino group is not terminal and is on the  $\alpha$ -carbon atom, steric hindrance arising in part from the combined interference of both the carboxyl group and the hydrocarbon chain extending beyond the  $\alpha$ -amino group may keep these charges farther apart, thereby reducing the attractive force between them.

Undoubtedly, as more data are collected some consideration could be given to the attractive forces of the negatively charged carboxyl groups of the amino acids already adsorbed by the soil and the effect of steric hindrance in adsorbing additional amino acid molecules containing only non-terminal  $\alpha$ -amino groups or the effect of lack of hindrance in adsorbing additional molecules with only terminal amino groups.

Undoubtedly, it will be found that other properties of the amino acids, and of the soil as well, will influence the attractive forces between the amino acids and the solid particles of the soil.

In Fig. 3 there might be some justification for putting the curve for unaltered cysteine, i.e., with zero time contact with the soil, parallel to and slightly above glycine. If this were its true position, replacing one of the H atoms on the  $\beta$ -carbon atom of  $\alpha$ -alanine with the SH radical, thereby forming cysteine, would increase the power of the soil to adsorb it. If instead of the SH radical an OH group had been

substituted, serine,  $\text{OHCH}_2\text{CHNH}_2\text{COOH}$ , would result. The power of adsorption of alanine was increased by this substitution but not as greatly as when SH was substituted. The substitution of a phenyl group for an H atom on the  $\beta$ -carbon atom of  $\alpha$ -alanine to make phenylalanine resulted in a weaker adsorption of it by the soil. The substitution of the imidazole group which has basic properties on the  $\beta$ -carbon to make histidine greatly increased the power of the soil to hold this molecule.

With the dicarboxylic amino acids, lengthening the chain as by changing aspartic acid to glutamic acid slightly reduced adsorption; changing one of the carboxyl ( $-\text{COOH}$ ) groups of aspartic acid to the carbamyl group ( $-\text{CONH}_2$ ) to make the amide, asparagine, resulted in an increased adsorption. This increase in adsorption following the replacement of a carboxyl with a carbamyl group is further evidenced if we assume that formic, acetic, and propionic acids are only very slightly, if at all, adsorbed by the soil, as formamide, acetamide, and propionamide are definitely, though only weakly, adsorbed (4).

In general, data obtained in this study agree qualitatively with preliminary studies using laboratory adsorbents. Some investigators have sought to separate the various amino acids by differential adsorption on colloids. Thus, Fuchs (8), using M/100 solutions of the different amino acids or of their chlorides in 5%  $\text{H}_2\text{SO}_4$ , found that the "acid" adsorbent, frankonite, adsorbed 98.5% of creatinine, and in decreasing amounts, arginine, histidine, ornithine, cystine, tyrosine, alanine, glutamic acid, cysteine, and aspartic acid down to 67.8% of glycine. By the same procedure, by using Lloyd's reagent, he secured slightly lower percentages of adsorption with the first four compounds and the same order except that arginine was more strongly adsorbed than creatinine. With four "neutral" adsorbents of the fuller's earth type he secured adsorption with arginine ranging from 78 to 86% and with glycine from 43 to 56%.

Mashino and Shikazono (10), secured from a 0.5% solution of the amino acids with the adsorbent, Japanese acid clay, adsorption of 3.35 to 11.14% of the monoamino acids, glycine, alanine, leucine, phenylalanine, glutamic acid, and aspartic acid, while with the diamino acids, or their chlorides, they secured 86.8% with arginine, 84.8% with histidine, and 62.9% with lysine. It is not entirely unexpected that the different adsorbents showed local differences or reversals in the general order of strength of adsorption with which the various amino acids are held, since the strength of adsorption of any amino acid on any adsorbent is undoubtedly dependent upon a number of the properties of each.

The data of Ensminger and Gieseking (7) indicate that proteins are partly adsorbed as cations by montmorillonitic clays. Undoubtedly cationic exchange was likewise partially responsible for the data obtained in this study.

#### SUMMARY

Preheating soil in an effort to inactivate any enzymes or other thermolabile catalysts in the soil had only a slight effect upon the ability of the soil to remove amino acids from percolating solutions.

Though some evidence was obtained in support of the existence of small but thermostable, small but thermolabile, and either a weak microbial activity or a catalyst inactivated by toluene as responsible for the removal of nitrogen from percolating solutions of amino acids, still the most important factor in removing amino acids from solutions was adsorption by the solid phase of the soil.

Adsorption isotherms of a number of the more soluble amino acids with preheated Yolo fine sandy loam were obtained by the usual laboratory methods of analyzing a series of equilibrium extracts from the soil. The dicarboxylic mono-amino acids, aspartic and glutamic, were most weakly adsorbed, the monocarboxylic mono-amino acids, glycine, alanine, leucine, and norleucine, were somewhat more strongly adsorbed, and the monocarboxylic amino acids with one or more nitrogen-containing radicals in addition to the  $\alpha$ -amino radical, such as lysine, histidine, and arginine, were most strongly adsorbed—even more strongly so than the  $\text{NH}_4$  ion of  $\text{NH}_4\text{Cl}$ .

Evidence was obtained which indicates that lengthening the hydrocarbon chain in the amino acids or transferring the  $\text{NH}_2$  radical farther out on the chain away from the carboxyl group increases adsorption; and that replacing a hydrogen on the terminal carbon atom of the chain with a phenyl group decreases adsorption, while an OH substitution increases the adsorption some and an SH substitution increases it somewhat more.

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## CONTAINERS FOR CORN MOISTURE SAMPLES<sup>1</sup>

JOE L. ROBINSON AND JOHN E. CHRISTENSEN<sup>2</sup>

**E**VEN though a sample of corn has been obtained with great care and accuracy, it will have little value for moisture determination purposes unless retained in a container which prevents the loss of moisture. Each fall many samples are obtained from different kinds of corn in yield comparisons, corn grown for seed, supplies handled commercially, or corn sealed for federal commodity loans. In some cases the moisture determination is not made for several days after the samples have been obtained. It is essential, therefore, that the container used be one which will prevent moisture losses from the time the samples are obtained until the moisture determination is made.

A study was undertaken by the Farm Crops Department at Iowa State College in the fall of 1938, and repeated in 1939, to determine the relative superiority of different types of containers for corn moisture samples.

### METHOD OF MAKING THE COMPARISON

Five types of containers were used in the fall of 1938. One type consisted of a pint metal can with a double friction lid; the second, the same container without the lid; third, a pint cardboard ice cream container; the fourth, a double cellophane bag with the top given one fold and fastened with wire paper clips; and the fifth, a double cellophane bag with the top folded twice and fastened with wire paper clips. With few exceptions the study with each type of container and with each level of moisture was repeated five times. Three hundred grams of corn were placed in each container and these were weighed at 24-hour intervals through a period of 14 days. The moisture content of the corn used in the fall of 1938 was raised by wetting it and placing in a tight container until the moisture content of the entire lot apparently had become uniform. The experiment was repeated with corn at three levels of moisture, namely, 18, 23, and 31%. The containers were arranged on a table in a laboratory, the temperature of which varied from 70° to 75° F. The humidity was quite low.

Two lots of corn, one containing 19% and the other 28% of moisture, were used in the fall of 1939. These were used as they came from the field without drying or adding moisture. The containers with the corn at the 28% moisture level were stored in two places. One set was handled in a manner similar to that used in 1938, while the other was placed in a room where the temperature varied from 40° to 60° and which had a much more humid atmosphere. The corn containing 19% moisture was stored under the same conditions as that used in 1938.

Only three types of containers were used the second year, namely, a metal can with a double friction lid, a cardboard ice cream container, and a double cellophane bag with the top folded twice.

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## RESULTS

In 1938 (Table 1) the 18% moisture corn in the metal cans with the cover in place lost no moisture during the 14 days of the experiment. However, the 23% moisture corn indicated a loss on the eleventh day, while the 31% corn indicated a loss on the third day.

TABLE 1.—Percentage of weight lost from corn that originally had 18, 23, and 31% moisture when held at room temperature in different types of containers for 14 days, 1938.

Type of container	Number of days in container						
	2	4	6	8	10	12	14
18% Moisture Corn							
Metal can, covered.....	0	0	0	0	0	0	0
Metal can, not covered.....	1.9	3.2	3.9	5.4	6.4	7.4	7.9
Ice cream container.....	2.1	3.6	5.7	7.1	8.7	10.1	11.2
Cellophane bag, one fold....	0	0	0	0.1	0.1	0.2	0.5
Cellophane bag, two folds....	0	0	0	0	0	0.1	0.1
23% Moisture Corn							
Metal can, covered.....	0	0	0	0	0	0.1	0.1
Metal can, not covered.....	1.4	2.4	3.2	4.1	5.1	6.3	7.1
Ice cream container.....	1.0	3.0	4.7	5.9	7.0	8.6	9.5
Cellophane bag, one fold....	0	0	0.2	0.5	0.9	1.0	1.2
Cellophane bag, two folds....	0	0	0	0.3	0.5	0.6	0.6
31% Moisture Corn							
Metal can, covered.....	0	0.2	0.4	0.5	0.7	0.7	0.9
Metal can, not covered.....	2.8	6.2	7.8	10.2	11.2	12.7	14.1
Ice cream container.....	1.4	4.6	6.7	9.0	10.6	12.6	14.7
Cellophane bag, one fold....	0	0.3	0.4	0.9	1.1	1.4	1.9
Cellophane bag, two folds....	0	0.1	0.5	0.9	0.9	1.2	1.9

It was evident that when corn with a high moisture percentage was placed in a container and stored at room temperature sufficient pressure developed to permit the escape of a small quantity of moisture even from a metal can with an apparently tight-fitting double friction lid. It is recognized that a portion of this indicated loss might have been due to respiration. The 31% corn lost more weight than the other two samples when placed in the open metal containers. It is of particular interest to note, however, that equally wet corn in an ice cream container lost water more rapidly than that held in the metal container with the lid removed. A feasible explanation of this is that moisture from the corn in the metal can was lost only from the exposed upper surface, while moisture loss was possible from the entire surface of the cardboard ice cream container. Little difference existed between the cellophane bags with one fold and two folds, although the bag with only one fold was the first of the two to show loss for each of the three moisture levels. The loss was progressively higher as the moisture percentage of the corn increased.

In 1939 (Table 2) the covered metal cans stored at room temperature permitted no moisture to escape when filled with 18% moisture corn and lost only 0.6% in 14 days when filled with 28% corn. The ice cream containers permitted moisture to escape from both lots, although more than twice as much was lost from the wetter corn. The 19% moisture corn placed in a cellophane bag lost only 0.1% of moisture in 14 days while 1.8% escaped from the 28% corn.

TABLE 2.—Percentage of weight lost from corn that originally had 19 and 28% moisture when held in different types of containers for 14 days, 1939.

Type of container	Number of days in container						
	2	4	6	8	10	12	14
19% Moisture Corn, Room Temperature							
Metal can . . . . .	0	0	0	0	0	0	0
Ice cream container . . . . .	1.3	2.1	2.9	3.4	4.1	4.8	5.4
Cellophane bag . . . . .	0	0	0.1	0.1	0.1	0.1	0.1
28% Moisture Corn, Room Temperature							
Metal can . . . . .	0	0	0.1	0.2	0.4	0.5	0.6
Ice cream container . . . . .	2.5	4.3	5.9	7.8	9.5	11.3	12.3
Cellophane bag . . . . .	0	0.1	0.3	0.7	1.0	1.4	1.8
28% Moisture Corn, Low Temperature							
Metal can . . . . .	+0.3	+0.3	+0.1	+0.1	+0.1	+0.1	+0.1
Ice cream container . . . . .	0.7	2.3	3.5	4.9	6.2	7.1	8.2
Cellophane bag . . . . .	+0.3	+0.1	0.4	0.4	0.7	0.9	1.1

When filled with 28% moisture corn and placed in the cooler and more humid atmosphere, the metal cans actually showed a slight gain in weight, due apparently to the condensation of moisture on the outside. The corn in the ice cream containers, however, lost a considerable portion of its moisture even under these storage conditions. The cellophane bags gained in weight at first, due probably to condensation, after which there was a very slight loss.

An additional set of ice cream containers filled with 28% moisture corn was packed in a shipping carton, much as they would be when shipped to the laboratory, to determine whether or not this additional protection might change the rate of loss. This, however, was not the case as the loss from the containers packed in cartons was approximately the same as from those not packed.

#### SUMMARY AND CONCLUSIONS

This study indicated that metal cans and cellophane bags are satisfactory for use in storing corn previous to making a moisture determination. The commonly used ice cream containers permitted the escape of moisture at the rate of 0.5 to 1.0% daily, depending upon the wetness of the corn. Such containers are not satisfactory and should not be used for this purpose.

The rate of moisture loss from each type of container was highest for those containing corn with the highest moisture percentage. The higher the percentage of moisture, the more necessary it becomes to have containers which are perfectly sealed.

A number of cardboard ice cream containers were packed in a carton such as is done in shipping. The rate of moisture loss from the packed containers differed little from the containers which were stored individually.

## VARIETAL REACTION TO BUNT IN THE WESTERN WHEAT REGION OF THE UNITED STATES<sup>1</sup>

C. S. HOLTON AND C. A. SUNESON<sup>2</sup>

COOPERATIVE uniform winter wheat bunt nurseries have been grown in the Intermountain and Pacific Coast states since 1930. Similar nurseries also were grown in the hard red winter wheat region.<sup>3</sup> Such nurseries indicate the bunt reaction of commercial varieties and promising hybrid material, as well as the prevalence of different bunt races in various parts of these areas.

This article summarizes the results obtained from 1932 to 1940 with 83 varieties and hybrid selections tested for bunt reaction during one or more years at 11 cooperating stations in the western states and at Kearneysville, W. Va., except that reaction to "dwarf" bunt is not included. The tests at Kearneysville, W. Va., were conducted as a check, under another environment, of the results obtained in the western states. The uniform bunt nurseries have been discontinued in the western region except in areas affected by "dwarf" bunt. In the future promising experimental material will be tested for reaction to each of the known races of bunt at Pullman, Wash., only.

### PREVALENCE OF BUNT IN THE WESTERN STATES

Bunt is the major disease of wheat in the Pacific Northwest and Intermountain states. It is more important in some sections than in others and is much more prevalent in fall-sown wheat than in spring wheat. Winter wheats have been damaged more by bunt in the Palouse region of southeastern Washington and northern Idaho, and more recently in Utah and southern Idaho than in any other part of the United States. Table 1 shows the number and percentage of the cars of wheat grading smutty at Pacific Northwest and Utah inspection points for the 19 marketing years 1922-23 to 1940-41, inclusive. The Pacific Northwest inspection points included the Columbia River, Puget Sound, and Spokane markets. The data were compiled from reports of the Agricultural Marketing Service.<sup>4</sup> The low percentage of smutty cars of grain reported for the Pacific Northwest in 1928 is attributable to the high proportion of spring wheat sown in consequence of the severe winter injury to fall-sown grain.

<sup>1</sup>Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, and the agricultural experiment stations of Arizona, Idaho, California, Montana, Oregon, Utah, Washington, and West Virginia. Received for publication August 2, 1941.

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<sup>3</sup>RODENHISER, H. A., and QUISENBERRY, K. S. Bunt reaction of some varieties of hard red winter wheat. *Jour. Amer. Soc. Agron.*, 30:484-492. 1938.

<sup>4</sup>Report of U. S. D. A. Agricultural Marketing Service, Federal Grain Supervision, Portland, Ore. Mimeographed. 1939.

TABLE 1.—Total carloads of wheat and the number and percentage grading smutty at North-Pacific and Utah inspection points for the marketing years 1921-22 to 1940-41, inclusive.

Marketing year	Pacific Northwest Inspection Points			Utah Inspection Points		
	Total car receipts, number	Carloads smutty, number	Smutty, %	Total car receipts, number	Carloads smutty, number	Smutty, %
1922-23.....	32,534	14,214	43.7	—	—	—
1923-24.....	55,537	23,022	41.5	4707	599	12.7
1924-25.....	35,154	17,717	50.4	3418	691	20.2
1925-26.....	41,662	11,567	27.8	6233	1786	28.7
1926-27.....	50,227	18,504	36.8	5359	1625	30.3
1927-28.....	65,543	22,945	35.0	7153	2317	32.4
1928-29.....	53,449	7,161	13.4	7116	2505	35.2
1929-30.....	49,413	10,448	21.1	6145	2858	46.5
1930-31.....	51,349	14,699	28.6	6220	2763	44.4
1931-32.....	40,140	14,713	36.7	4320	997	23.1
1932-33.....	36,607	8,408	23.0	4193	1037	24.7
1933-34.....	47,677	10,555	22.1	3513	602	17.1
1934-35.....	36,778	7,352	20.0	4002	989	24.7
1935-36.....	43,822	10,266	23.4	5319	2115	39.8
1936-37.....	47,746	7,953	16.7	4446	720	16.2
1937-38.....	49,166	4,011	8.2	4950	839	16.9
1938-39.....	66,009	5,004	7.6	6586	1930	29.3
1939-40.....	48,017	2,701	5.6	4584	578	12.6
1940-41.....	—	—	8.6*	—	—	17.4*

\*Estimate based on receipts for 7-month period, August, 1940, to February, 1941, inclusive.

The general decline in bunt, particularly in the north-Pacific area, has been very encouraging, especially if it can be assumed that the increase indicated by the preliminary figures for 1940 is merely sporadic. The decline in smut damage probably can be attributed to the release of resistant varieties and to more effective control measures. The use of copper carbonate dust for bunt control in the region began about 1922.<sup>5</sup> Other copper dusts and organic mercury dusts were used later. The resistant varieties that contributed materially to the decline of bunt with the year of their release for commercial growing are as follows: Rident (1924); Sherman (1926); Oro (1927); Albit (1927); Rio (1930); Relief (1931); Yogo (1933); Rex (1933); and Hymar (1935).

## MATERIALS AND METHODS

The wheats included in the uniform winter wheat bunt nursery tests were the common commercial varieties of the region, varieties used in physiologic race identification,<sup>6</sup> and hybrid selections which appeared to have value either as potential commercial varieties or as parent stocks. The susceptible varieties

<sup>5</sup>HEALD, F. D., and SMITH, L. J. The dusting of wheat for bunt or stinking smut. Wash. Agr. Exp. Sta. Bul. 171. 1922.

<sup>6</sup>RODENHISER, H. A., and HOLTON, C. S. Physiologic races of *Tilletia tritici* and *T. levis*. Jour. Agr. Res., 55:483-496. 1937.

Hybrid 128 and Kharkof were included in order to determine whether the environmental conditions were favorable for infection. Each variety was grown in duplicate rows at each station, the length of the rows varying from 5 to 16 feet at the various stations.

Prior to inoculation the seed was soaked 5 minutes in a 1-320 solution of formaldehyde, after which the formaldehyde was washed off with running water and the seed allowed to dry. Approximately 5-gram lots of seed for a single row of a variety were then placed in coin envelopes. The seed was then inoculated by adding sufficient chlamydospores to each envelope to insure a visible blackening of all of the kernels after agitating the envelopes. The inoculated seed was sent to the cooperators at the various stations where it was planted at whatever time environmental conditions presumably were favorable for infection.

The inoculum used for each nursery, except at Corvallis, Ore., and Kearneysville, W. Va., was collected from commercial wheat fields in the vicinity of the particular nursery and presumably it contained a composite of the races of *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn prevalent in those areas. The inoculum for the nursery at Corvallis, Ore., was obtained from commercial wheat fields of that vicinity for the 1932 and 1933 tests, but for the 1934 test the inoculum was a composite of collections from the smutted varieties in the 1933 nursery. In subsequent years the seed for the Corvallis and Kearneysville nurseries was inoculated with a composite of spores from the entire Pacific Northwest, except that for 1934 the seed for Kearneysville was inoculated with spores from the same source as those for the Pullman, Wash., nursery.

The percentages of infection were determined on the basis of total and smutted heads in a row. During the first six years of the tests, the bunt percentages were obtained by actual counts of the heads. In 1938, however, the percentages were determined by estimation except in rows having less than 10% smutted heads, in which case head counts were made. This method was also used for some of the nurseries in 1939, while in others in that year and in all nurseries in 1940 all percentages were estimated except for occasional random rows in which counts were made for checking purposes. The estimates were found to be sufficiently accurate.

Varieties which showed a high degree of susceptibility, or that were otherwise unsatisfactory, were eliminated from the nurseries and new varieties were added from time to time. Consequently, the number of varieties was not constant from year to year, nor were all of the varieties tested the same number of years. At the end of each season the data obtained from the nurseries were summarized in mimeograph form for distribution to the cooperators and other interested individuals.

#### EXPERIMENTAL RESULTS

Table 2 shows the average percentage of bunt on each variety at each station and the number of years each was tested.<sup>7</sup> The varieties are listed in the descending order of their resistance, based on the weighted average percentage of bunt obtained.

The data presented in Table 2 reveal several notable facts. None of the 83 varieties tested was bunt-free at all of the stations. However, 36 varieties averaged less than 10% bunt and 7 averaged less than 1%. The remainder showed varying degrees of susceptibility,

<sup>7</sup>Data from nurseries grown at Newton and Avon, Utah, and at Lind, Wash., for one year and at Tucson, Ariz., for five years are not included because of low infection percentages or poor stands.

TABLE 2.—Average bunt reaction of winter wheat varieties in the western

Variety	C. I. No.	Years tested and average percentage of bunt									
		Pullman, Wash.		Pendleton, Ore.		Moro, Ore.		Corvallis, Ore.		Moscow, Ida.	
		No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %
Hussar X Hohenheimer.....	10068-1	7	0.1	7	0.2	4	0.0	4	0.1	7	0.8
Relief X Ridit.....	11908	1	0.0	1	0.0	1	0.0	—	—	1	2.0
Relief X Ridit.....	11905	3	0.0	3	0.3	3	0.0	—	—	3	1.2
Oro X Turkey Florence.....	11865	4	0.0	4	0.0	3	0.8	1	0.0	4	0.2
Oro X Turkey Florence.....	11864	2	0.0	2	0.0	1	0.0	1	2.1	2	0.0
Relief X Ridit.....	11904	3	0.0	3	0.3	2	0.5	—	—	3	1.6
Ridit X Hohenheimer.....	11760	5	0.8	5	0.3	3	0.6	2	0.0	5	1.0
Jenkin X Ridit.....	10081	6	2.1	6	0.8	4	0.8	6	1.3	6	1.3
Hohenheimer*.....	11458	8	0.6	8	7.5	5	1.5	5	0.0	8	0.1
Ridit X Utah Kanred.....	11598	4	5.3	4	2.3	3	1.2	4	3.6	4	1.6
Hussar X Hohenheimer.....	10068	1	0.2	1	3.7	1	0.0	1	4.6	1	5.2
Hohenheimer X Goldcoin.....	11098	3	2.7	3	11.5	2	2.5	3	0.6	3	0.1
Ridit X White Odessa.....	11449	5	5.2	5	0.1	3	0.1	5	5.0	5	3.2
Relief X Ridit.....	11909	2	2.2	2	0.1	2	1.2	—	—	2	3.0
Ridit X Utah Kanred.....	11601	1	12.3	1	11.6	1	0.0	1	0.0	1	0.0
Ridit X Utah Kanred.....	11599	4	5.0	4	5.9	3	0.6	4	6.7	4	1.4
Ridit X Utah Kanred.....	11086	3	4.3	3	5.4	2	0.5	3	6.8	3	4.1
Ridit X Utac.....	11604	3	10.3	3	3.1	2	0.3	3	4.5	3	3.0
Hosar.....	10067	4	3.5	4	18.0	3	4.5	4	0.6	4	0.5
Turkey X Florence.....	10080	5	9.0	5	6.0	3	0.2	5	6.6	5	4.6
Ridit X Utah Kanred.....	11600	1	9.9	1	19.5	1	2.6	1	0.8	1	0.0
Ridit X Utah Kanred.....	11597	3	7.6	4	7.1	3	1.1	4	3.9	4	2.3
Ridit X Utah Kanred.....	11696	2	6.9	2	6.0	1	3.6	2	5.1	2	2.4
Beloglina X Hussar.....	11513	6	5.9	6	3.8	4	1.7	3	13.7	6	14.3
Ridit.....	6793	9	10.6	9	3.2	6	1.8	6	5.5	9	9.1
Relief*.....	10016	9	11.2	9	4.0	6	0.3	6	16.6	9	7.1
Turkey selection.....	7366	6	2.3	6	3.4	4	2.7	6	7.3	6	3.0
Turkey selection.....	11603	5	2.1	5	4.9	3	0.6	5	10.2	5	3.0
Ridit X Sevier selection.....	11603	1	24.5	1	11.0	1	1.8	1	11.4	1	0.0
Turkey X Bearded Minnesota No. 48.....	8243	5	9.0	5	11.3	3	3.2	5	6.0	5	5.7
Ridit X Utah Kanred.....	11687	2	12.8	2	18.9	2	10.7	2	10.2	2	6.2
Hussar X Turkey.....	11596	1	26.0	1	0.7	1	0.0	1	53.2	1	0.9
Minturki.....	6155	9	8.3	9	20.4	6	8.0	6	2.4	9	17.1
Hussar*.....	4843	9	13.7	9	5.2	6	0.9	6	21.0	9	15.5
Turkey selection.....	11370	6	6.6	6	10.4	4	7.2	6	12.4	6	8.3
Hussar X Turkey.....	11444	2	37.5	2	6.5	1	2.5	2	11.0	2	9.7
Turkey selection.....	11424-14	2	1.0	2	0.5	2	28.2	—	—	2	0.5
Turkey selection.....	1532	5	6.0	5	9.5	3	3.3	5	17.9	5	8.0
Oro*.....	8220	9	4.5	9	2.0	6	21.7	6	12.6	9	4.3
Oro X Hybrid 128.....	11758	4	1.2	4	1.3	2	28.6	2	18.1	4	2.0
Kanred X Hussar.....	11445	2	38.6	2	6.0	1	0.0	2	19.9	2	9.2
Turkey selection.....	11530	7	13.8	7	9.5	4	4.6	4	22.6	7	34.3
Hussar X Turkey.....	11443	2	33.6	2	19.1	1	10.2	2	6.2	2	19.8
Turkey X Hohenheimer.....	11759	4	1.1	4	1.3	2	34.1	2	21.6	4	1.3
Ridit X Pacific Bluestem.....	11450	4	15.5	4	23.9	3	3.3	4	14.9	4	17.9
(Hybrid 128 X White Odessa) X Utah Kanred.....	11602	2	38.5	2	0.0	2	4.9	2	46.7	2	6.7
Rio.....	10061	9	2.1	9	2.6	6	24.6	6	23.8	9	2.6
Oro X Hybrid 128.....	11756	4	1.0	4	9.4	2	33.2	2	43.4	4	0.5
Hussar X Turkey.....	11527	1	41.6	1	10.2	—	—	1	24.0	1	4.4
(Turkey X Hybrid 128) X Hussar.....	11699	2	16.7	2	0.3	1	6.8	2	39.8	2	13.0
Oro X Hybrid 128.....	11757	4	0.8	4	0.5	2	37.9	2	37.4	4	0.8
Brevon.....	11912	1	6.0	1	18.0	1	1.0	—	—	1	2.0
Turkey selection.....	11424-8	2	1.5	2	0.8	2	46.2	—	—	2	0.1
Ashkof.....	6680	2	29.6	2	31.3	1	0.0	2	6.0	2	11.5
Oro X Federation.....	11914	1	1.0	1	2.0	1	48.0	—	—	1	4.0
Martin.....	4463	8	32.7	8	8.7	5	7.8	5	34.0	8	36.7
Hussar X Turkey.....	11528	1	60.3	1	21.6	—	—	1	20.5	1	7.0
Martin X (Sevier X Dicklow) (Hybrid 128 X White Odessa) X Utah Kanred.....	11446	1	78.0	1	3.0	1	4.5	1	24.1	1	19.5
Turkey selection.....	11529	1	38.5	1	23.6	—	—	1	36.0	1	5.6
Beloglina.....	10015	2	27.3	2	32.5	1	43.1	2	6.4	2	38.8
Sherman.....	1543	1	29.8	1	36.7	1	23.2	1	9.7	1	45.9
Hymar.....	4430	2	82.2	2	18.7	1	3.4	2	31.4	2	20.4
Martin X (Sevier X Dicklow).....	11605	7	39.2	7	11.1	5	11.2	4	65.4	7	49.7
Rex M1.....	11448	2	76.5	2	16.6	1	2.7	2	37.7	2	22.7
	11689	6	33.6	6	13.1	4	9.4	3	41.4	6	63.8

wheat region and at Kearneysville, W. Va., 1932 to 1940, inclusive.

Years tested and average percentage of bunt

NezPerce, Ida.	Rockland, Ida.	Malad, Ida.	Tetonia, Ida.	Logan, Utah	Bozeman, Mont.	Kearneys- ville, W. Va.	Wgtd. aver- age of bunt, %	Total sta- ys.
No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %	
6	0.0	5	0.0	3	0.0	6	0.0	63
1	0.0	1	0.0	1	0.0	1	0.0	9
3	0.0	3	1.0	3	0.0	3	0.0	28
4	0.0	3	0.0	3	0.1	2	0.2	36
2	0.0	1	0.0	1	0.0	2	1.5	17
3	1.5	3	0.6	3	0.0	3	0.5	27
5	1.6	4	1.2	3	0.3	4	0.2	46
3	2.5	4	0.5	5	0.1	4	0.1	53
6	1.5	6	0.0	3	0.0	5	0.1	72
3	3.8	3	0.5	3	0.8	4	0.8	40
—	—	1	1.3	—	1.3	—	—	7
3	1.9	2	0.0	—	0.1	3	0.4	29
2	2.5	4	0.0	5	2.2	3	3.1	44
2	9.0	2	4.7	2	0.0	2	0.1	10
—	—	1	0.0	1	0.0	1	0.0	10
3	3.1	3	1.1	3	0.2	4	2.9	40
3	3.0	2	0.1	—	4.1	3	2.7	30
2	2.3	3	1.6	—	2.5	3	5.1	31
1	15.2	3	0.0	—	0.1	2	1.6	34
2	5.1	4	1.7	—	1.7	3	2.7	44
—	—	1	0.0	—	0.0	1	1.3	10
3	7.1	3	1.1	—	3	3.1	4	39
2	3.3	2	5.4	—	4.3	2	8.7	21
6	3.4	5	1.2	3	4.4	5	3.0	56
6	5.7	7	2.7	3	4.0	8	3.2	80
6	4.7	7	1.3	3	3.8	8	2.0	80
3	13.8	4	17.8	—	8	2.7	4	48
2	6.5	4	10.9	—	5	3.2	2	43
—	—	1	9.2	—	1	0.0	1	10
—	—	—	—	—	—	—	—	—
2	2.8	4	15.8	—	5	4.1	3	44
2	8.8	2	7.2	—	2	5.9	2	22
—	—	1	3.0	—	1	0.0	1	10
6	6.4	7	7.6	3	2.7	8	5.4	79
6	8.7	7	1.0	3	5.1	8	4.3	80
3	10.4	4	22.4	—	5	5.6	3	52
—	—	1	0.8	—	2	2.8	—	13
2	1.0	2	21.5	2	7.7	2	1.1	10
2	6.0	4	30.8	—	5	4.8	2	43
6	2.4	7	35.6	3	36.0	8	5.2	80
4	1.7	3	55.8	2	30.2	3	3.8	37
—	—	1	3.5	—	2	4.7	—	13
6	19.8	1	0.2	3	7.5	6	5.0	13
4	3.5	3	38.1	2	38.7	3	3.6	37
1	6.3	3	10.7	—	4	9.0	2	34
—	—	—	—	—	—	—	—	—
1	33.6	2	1.1	—	2	13.4	2	21
6	1.7	7	41.3	3	38.3	8	1.0	79
4	1.8	3	54.1	2	40.0	3	2.3	37
—	—	—	—	—	1	0.0	—	6
2	16.1	2	0.5	—	2	21.6	2	21
4	1.8	3	50.4	2	43.7	3	1.5	37
1	57.0	1	15.0	1	24.5	1	20.0	9
2	1.2	2	31.7	2	15.5	2	0.2	19
2	29.9	1	1.1	—	1	6.4	2	19
1	4.0	1	1.0	1	0.5	1	18.9	9
6	42.6	6	5.4	3	5.4	7	2.2	72
—	—	—	—	—	1	0.2	—	6
—	—	1	10.0	—	1	2.3	—	7
—	—	—	—	—	1	0.2	—	6
—	—	1	5.6	—	2	9.4	—	13
—	—	1	7.7	—	1	3.7	—	7
—	—	1	0.7	—	2	2.7	—	13
6	36.6	6	1.8	3	17.9	6	5.9	60
—	—	1	2.2	—	2	8.1	—	13
6	53.4	5	0.6	3	7.4	5	7.7	57



TABLE 2.—

Variety	C. I. No.	Years tested and average percentage of bunt									
		Pullman, Wash.		Pendleton, Ore.		Moro, Ore.		Corvallis, Ore.		Moscow, Ida.	
		No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %
Rex.....	10065	9	47.9	9	13.5	6	8.9	5	46.1	9	49.9
Hybrid 128 X Martin.....	11606	3	60.2	3	1.1	2	21.4	3	74.0	3	12.6
Albit*.....	8275	8	56.1	8	15.3	5	15.4	6	49.7	8	38.6
Dickkopf X Silvercoin.....	11697	1	44.9	1	88.1	1	25.9	1	59.0	1	15.1
Hard Federation X Martin.....	11691	2	48.3	2	2.8	1	34.0	2	58.2	2	24.5
Oro X Federation.....	11692	3	53.2	3	11.1	2	25.3	3	51.3	3	24.6
Hybrid 128 X White Odessa.....	11913	1	1.5	1	2.0	1	58.0	—	—	1	2.0
Hard Federation X Martin.....	11607	3	68.0	3	4.6	2	43.3	3	64.5	3	19.4
Hard Federation X Martin.....	11488	3	64.7	3	4.8	2	13.9	3	75.3	3	23.9
Martin X (Sevier X Dicklow).....	11447	2	87.7	2	12.2	1	6.3	2	56.8	2	32.1
White Odessa.....	4955	6	65.0	6	16.3	4	11.7	6	58.8	6	25.2
Rex M2.....	11690	2	57.9	2	1.8	1	33.8	2	67.3	2	38.4
Turkey selection.....	6175	7	42.4	7	10.9	4	40.0	5	61.5	7	28.2
Turkey selection.....	11424	1	88.8	1	3.0	—	—	1	70.4	1	58.3
Triple X White Odessa.....	11688	1	59.0	1	3.7	1	62.4	1	87.3	1	45.7
Minhardt.....	5149	1	87.5	1	70.0	—	—	1	51.5	1	73.1
Kharkot.....	1442	9	71.0	9	42.3	6	60.4	6	72.3	9	63.3
Hybrid 128*.....	4512	8	81.1	8	91.6	6	80.3	5	81.8	8	81.8

\*Varietal host testers used in physiologic race identification, see footnote 7.

ranging from 10.8 to 73.7%. Among the common commercial varieties of the region only Redit and Relief had less than 10% infection, and it seems significant that one or both of these varieties entered into the production of 17 of the hybrid selections in this low-infection group. Hussar and Hohenheimer, two varieties not of commercial importance but having less than 10% infection, entered into the production of seven other hybrid selections in the low infection group. Among these seven were Hosar and also Hussar X Hohenheimer (C. I. 10068-1), the most resistant of all the varieties tested. Turkey-Florence (C. I. 10080), a sister selection of Redit and apparently identical in smut reaction, also is in this group and this variety entered into the production of two of the highly resistant selections. Thus 26 of the 27 most highly resistant selections were from hybrids involving one or more of the five resistant varieties mentioned above. On the other hand, all selections that had more than 10% bunt were from hybrids in which at least one susceptible parent was used. These results, therefore, emphasize again the importance of using bunt-resistant parents in the breeding of new wheat varieties for regions where this disease is prevalent.

The possibility of even greater resistance as a result of genetic recombinations is also evident in Table 2. Some of these recombinations, like the seven selections averaging less than 1% bunt, might have been anticipated from the available information on race reaction or genetic reaction of the parents involved. Favorable recombinations in resistant X susceptible crosses may also occur, however, as is evident in Jenkin X Redit (C. I. 10081) and in Redit X Utah Kanred (C. I. 11598).

Considered from the standpoint of individual stations the data in Table 2 present a somewhat different and more variable picture

*Concluded.*

Years tested and average percentage of bunt

NezPerce, Ida.		Rockland, Ida.		Malad, Ida.		Tetonia, Ida.		Logan, Utah		Bozeman, Mont.		Kearneys- ville, W. Va.		Wgtd. aver- age of bunt, %	To- tal sta- tions yrs.
No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %	No. years tested	Bunt %		
6	56.9	7	6.2	3	8.8	8	7.2	7	21.2	5	22.7	5	31.7	27.5	79
2	55.5	3	5.7	—	—	3	9.2	3	24.6	3	6.5	3	43.3	27.9	31
5	56.3	6	3.1	2	11.5	7	6.1	6	18.1	5	15.3	5	36.6	28.0	71
1	59.4	1	11.1	—	—	1	6.1	1	0.0	1	5.5	1	4.0	29.0	11
2	60.9	2	2.8	—	—	2	18.0	2	34.2	2	0.0	2	43.2	29.5	21
3	59.2	2	2.8	—	—	2	14.3	3	35.8	3	5.1	3	42.6	30.2	30
1	13.0	1	35.0	1	65.0	1	18.0	1	85.0	—	—	—	—	31.1	9
2	64.9	3	12.7	—	—	3	12.1	3	25.5	3	3.5	3	43.4	31.5	31
2	72.5	3	4.9	—	—	3	19.3	3	30.6	3	5.0	3	40.0	31.6	31
—	—	1	7.4	—	—	2	6.3	—	—	—	—	1	24.7	33.0	13
3	60.9	4	6.3	—	—	5	12.1	4	33.2	4	12.9	5	54.5	33.3	53
2	74.7	2	2.5	—	—	2	16.5	2	37.5	2	9.9	2	48.9	35.5	21
5	17.0	5	45.7	1	60.0	6	36.9	5	30.9	4	40.0	5	34.0	37.0	61
1	38.3	1	45.0	—	—	1	55.1	1	66.4	1	0.8	1	35.2	46.1	10
1	57.8	1	3.1	—	—	1	45.8	1	80.7	1	0.5	1	82.5	48.1	11
—	—	—	—	—	—	1	32.8	—	—	—	—	1	16.2	55.2	6
6	62.6	7	64.1	3	60.2	8	59.5	6	59.0	5	48.0	5	65.1	60.6	79
6	70.2	7	56.3	3	58.0	6	64.1	7	64.0	5	64.0	4	78.1	73.7	73

than when analyzed on the weighted average percentage basis. As shown in Table 3, some varieties were bunt-free at certain stations, the number of such varieties ranging from 2 at Kearneysville, W. Va., to 11 at Tetonia, Idaho. At eight of the stations the majority of the varieties had less than 10% infection, while at three stations a majority of the varieties had more than 10% infection. At Malad, Idaho, the number of infected varieties having more or less than 10% of infection was equal. At the three of the stations at which higher infections prevailed either conditions were more favorable for infection or different physiologic races comprised the inoculum.

TABLE 3.—Number of varieties falling into different bunt-infection classes at each station.

Station	Number of varieties			
	Bunt-free	Trace to 10%	With more than 10%	Total
Pullman, Wash. ....	5	34	44	83
Pendleton, Ore. ....	3	48	32	83
Moro, Ore. ....	9	41	28	78
Corvallis, Ore. ....	4	23	47	74
Moscow, Idaho. ....	4	46	33	83
Nez Perce, Idaho. ....	5	33	27	65
Rockland, Idaho. ....	10	46	23	79
Malad, Idaho. ....	6	14	14	34
Tetonia, Idaho. ....	11	55	17	83
Logan, Utah. ....	6	37	26	69
Bozeman, Mont. ....	5	45	15	65
Kearneysville, W. Va. .	2	33	36	71

The range of varietal susceptibility was greater at Corvallis, Ore., than at any other nursery location. This is the only nursery in which as many as 47 varieties had more than 10% smut. No doubt this is attributable, at least in part, to a greater representation of physiologic races in the inoculum for this station which, for the last two years the tests were made, was a composite from the entire Pacific Northwest. On the same basis, similar results might have been expected from the Kearneysville, W. Va., nursery. However, as shown in Table 3, only 36 varieties had more than 10% bunt, and since the inoculum for this nursery every year except one was a composite from the entire Pacific Northwest, it would appear that environmental factors were less favorable for bunt development at Kearneysville than at Corvallis.

Some indication of the predominant races in the inoculum is provided by the reaction of the host testers<sup>8</sup> at each station. For example, at Pullman, Wash., the susceptibility of Albit and varieties of similar reaction, such as Rex, Martin, White Odessa, and Hymar, indicates a predominance of inoculum of those races that are distinguished by their virulence on these varieties. These races appeared to be prevalent also at Corvallis, Ore., and Moscow and Nez Perce, Idaho. On the other hand, the results at Moro, Ore., and Rockland, Idaho, suggest a predominance of inoculum of the Oro race, whereas those at Malad, Idaho, indicate the presence of not only the Oro race but also races which infect other Turkey-type wheats. The results from the other stations indicate that the inoculum was made up either of relatively nonvirulent races or of inadequate representation of highly virulent races. For the most part, however, it appears that the more virulent races are fairly prevalent in the localities where these nurseries were grown. In any case these results emphasize the desirability for determining varietal resistance under a wide range of conditions in order to determine varietal adaptability to different localities, at least from the standpoint of bunt resistance. Obviously, however, results from tests of this type should be fortified by results from tests with individual races in order that the reaction of possible new varieties to all known races may be definitely determined. The importance of this in connection with the release and distribution of new varieties was recently pointed out by Rodenhiser and Quisenberry.<sup>9</sup>

#### SUMMARY

The reaction of 83 varieties and hybrid selections of winter wheat to bunt was determined by growing them in uniform nurseries at 11 localities in the western United States and at Kearneysville, W. Va., and inoculating them with spores obtained from commercial wheat fields in the vicinity of each nursery location, or from a composite of collections from commercial wheat fields in the entire Pacific Northwest.

None of the varieties was bunt-free at all of the stations, although 36 varieties averaged less than 10% bunt and 7 averaged less than

<sup>8</sup>See footnote 6.

<sup>9</sup>See footnote 3.

1%. Five resistant varieties were involved in the production of 26 of the 27 most highly resistant hybrid selections.

In 8 of the 12 nurseries the majority of the infected varieties and selections had less than 10% bunt.

The prevalence and distribution of race groups of the bunt fungi were indicated by the reaction of the host testers included in the nurseries each year. One or more of the more virulent races appear to be fairly prevalent throughout the western region.

BREEDING FOR DISEASE RESISTANCE IN OATS<sup>1</sup>H. C. MURPHY, T. R. STANTON, AND F. A. COFFMAN<sup>2</sup>

THE two smuts and two rusts affecting oats, namely, loose smut (*Ustilago avenae* (Pers.) Jens.), covered smut (*U. levis* (Kell. and Sw.) Magn.), crown rust (*Puccinia coronata avenae* Eriks.), and stem rust (*P. graminis avenae* Eriks. and Henn.), apparently have caused more damage to the oat crop in the United States than all other oat diseases combined. Among the minor oat diseases that frequently have caused considerable damage are oat blast (non-parasitic), Fusarium blight or foot rot (*Fusarium culmorum* (W.G.Sm.) Sacc., and other species), halo blight (*Bacterium coronafaciens* Ell.), stripe blight (*B. striafaciens* Ell.), Helminthosporium leaf blotch (*Helminthosporium avenae* Eidam), Pythium root necrosis (principally *Pythium debaryanum* Hesse), and others.

Epiphytotics of the two smuts vary considerably in severity from year to year and from region to region, but on the whole tend to be much more uniform in this respect than either crown or stem rust. Except for the smuts, it is difficult to obtain an accurate estimate of the damage caused by oat diseases. Estimates of losses caused by the rusts and minor diseases of oats as reported by the Plant Disease Reporter<sup>3</sup> are inadequate because of the evident lack of sufficient and consistent observations and the absence of any reliable basis for determining the actual losses caused by these diseases. Some idea of their relative importance in recent years is indicated, however, by the estimated reduction in Iowa oat yields for the years 1935 to 1939 (Table 1) and for Iowa, Minnesota, Illinois, and Wisconsin in the 5-year period 1935 to 1939 (Table 2).

In these leading oat-producing states, crown rust, smut, blast, foot rot, and stem rust, in the order named, apparently have been most important in recent years. Similar estimates during the 20-year period 1919 to 1938 would indicate, however, that smut, crown rust, and stem rust, in the order named, were most important for the same states and for the United States as a whole. The apparent decreasing relative importance of oat stem rust doubtless is due, in part, to the increasing acreage of stem-rust-resistant varieties, particularly in the Corn Belt. Probably the losses caused by some of the so-called minor diseases, such as oat blast, foot rot, Pythium root necrosis, etc., were

<sup>1</sup>Contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, and the Iowa Agricultural Experiment Station, Ames, Iowa, cooperating. Journal Paper No. J-837 of the Iowa Agricultural Experiment Station. Project No. 654. Also presented by the senior writer at the sessions of the Crops Section of the American Society of Agronomy, Chicago, Ill., December 5, 1940. Received for publication August 7, 1941.

<sup>2</sup>Pathologist, Senior Agronomist, and Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, respectively. Acknowledgment is hereby made to agronomists and pathologists of state and federal agricultural experiment stations and to other research institutions, many of which are named in Table 4, for contributing data on the disease resistance and agronomic value of the oat varieties and strains catalogued in this paper.

<sup>3</sup>Crop losses from plant diseases in the United States. U. S. D. A. Bur. Plant Ind. Plant Dis. Rptr. Sup. 94, 1936; 100, 1937; 108, 1938; 118, 1939; 127, 1940.

not recognized as such until the results of more extensive studies of these particular diseases became available.

TABLE 1.—Estimated reductions in Iowa oat yields caused by oat diseases during the 5-year period 1935 to 1939.\*

Diseases	Estimated reduction in average yield for Iowa, %					
	1935	1936	1937	1938	1939	5-year av.
Crown rust.....	20.0	Trace	1.0	24.0	3.5	11.6
Smut.....	1.0	2.0	5.0	4.0	5.0	3.4
Stem rust.....	1.0	0.5	0.5	0.5	Trace	0.5
Blast.....	—	—	0.5	0.5	0.5	0.5†
Foot rot.....	—	—	0.5	0.5	1.0	0.6‡
Root rot†.....	—	—	—	12.0	6.0	9.8§
All diseases.....	28.0	5.5	9.0	41.7	23.1	23.6

\*See footnote 3.

†Primarily *Pythium debaryanum*.

‡Average for 1937, 1938, and 1939.

§Average for 1938 and 1939.

TABLE 2.—Estimated reductions in Iowa, Minnesota, Illinois, and Wisconsin oat yields caused by oat diseases during the 5-year period 1935 to 1939.\*

Diseases	Estimated reduction in average yield 1935 to 1939, %			
	Iowa	Minn.	Ill.	Wis.
Crown rust.....	11.6	2.0	2.2	7.8
Smut.....	3.4	2.6	2.8	5.9
Stem rust.....	0.5	0.3	2.2	0.1
Blast.....	0.5†	—	13.9‡	4.5
Foot rot.....	0.6‡	Trace‡	—	—
Root rot†.....	9.8§	—	—	—
All diseases.....	23.6	5.1	21.6	14.0

\*See footnote 3.

†Primarily *Pythium debaryanum*.

‡Average for 1937, 1938 and 1939.

§Average for 1938 and 1939.

||Average for 1937 and 1938.

Statistical studies (6, 8, 15)<sup>4</sup> of the effect of crown and stem rust infections on oat yields indicate that some of the estimates of losses may have been too low; for example, the estimated reduction in Iowa oat production caused by crown rust in 1938 was 24%, or approximately 80 million bushels. On the basis of a statistical study of 442 oat varieties and selections grown in replicated tests at Ames and Kanawha, Iowa, in 1938, the simple regression for yield on crown rust was  $-0.44$  (Figs. 1 and 2); that is, for each unit increase in coefficient of crown rust infection, the average yield was reduced 0.44 bushel per acre. Because resistant varieties were not yet available, the entire Iowa oat acreage in 1938 obviously was sown with varieties moder-

<sup>4</sup>Numbers in parenthesis refer to "Literature Cited", p. 88.

ately to completely susceptible to crown rust. Assuming an average crown rust coefficient of 50, which on the basis of numerous observations would be very conservative, the reduction in Iowa oat yields in 1938 caused by crown rust would amount to 22 bushels per acre, or approximately 130 million bushels. With an actual state yield of 33.5 bushels per acre and a potential yield of 55.5, the loss from crown rust would have been 40% rather than the estimated 24%.

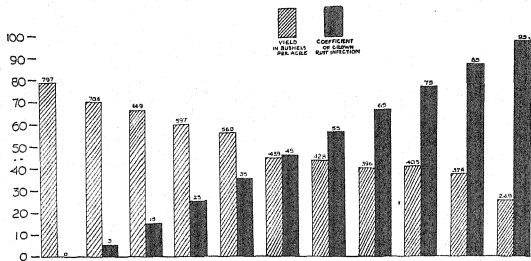


FIG. 1.—Distribution of average yields of 442 varieties and selections of oats according to coefficient of crown rust infection at Ames and Kanawha, Iowa, in 1938.

Marked progress has been made, and is being made, in the development of desirable agronomic oat varieties possessing combined resistance to the major oat diseases, i.e., the smuts and rusts. Less effort has been given toward the development of varieties resistant to the minor diseases. In comparison with the high degrees of resistance to the smuts and rusts now available, it appears that, in general, the available resistance to the minor diseases will be somewhat less efficient. The resistance now known may be sufficient, however, for adequate protection under most field conditions, and it is probable that a more extensive search will disclose sources of greater resistance, or even immunity, to one or more of the minor diseases.

#### DISEASE-RESISTANT OAT VARIETIES USED AS PARENTS IN BREEDING PROGRAMS

Fortunately, there is now available a source of adequate resistance to each of the races of the smuts, crown rust, and stem rust known to occur in North America. So long as this situation exists, breeding for resistance to the major oat diseases has a promising outlook. Some of the oat varieties that have been used most often as sources for resistance to the smuts, crown rust, and stem rust are presented in Table 3. Now that new agronomically desirable varieties with combined resistance to the major diseases are becoming available, they doubtless will replace many of these older varieties in future breeding programs.

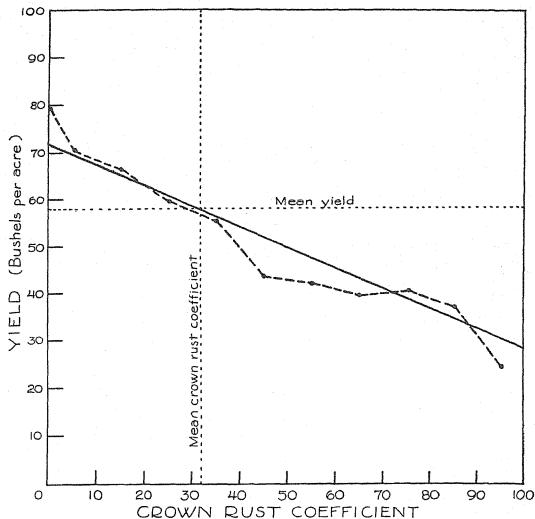


FIG. 2.—Simple regression of yield on coefficient of crown rust in 442 oat varieties and selections grown at Ames and Kanawha, Iowa, in 1938.

#### VARIETIES RESISTANT TO SMUTS

Reed (22) recently described 29 parasitic races of loose smut and 14 of covered smut. He found Victoria, Markton, Navarro, and Black

TABLE 3.—Disease-resistant oat varieties used as parents in breeding programs.

Varieties outstanding for resistance to					
Smut		Crown rust		Stem rust	
Variety name	Relative resistance	Variety name	Relative resistance	Variety name	Relative resistance
Victoria.....	++++*	Bond.....	+++	Richland.....	+++
Markton.....	+++	Victoria..	+++	Iogold.....	+++
Navarro.....	+++	Rainbow..	+	Rainbow.....	+++
Bond.....	++	Alber.....	+	White Tartar..	++
Black Mesdag..	++	Capa.....	+	Jostrain.....	+

\*++++=Highly resistant to all known races; +++=Highly resistant to all races tested or except to certain rare races; ++=Adequate resistance under most field conditions; +=Moderately resistant.



Mesdag resistant to all his races of loose smut, and Victoria and Markton resistant to all his races of covered smut, although an occasional infected plant of Markton was recorded. Unpublished data covering 66 station years obtained from uniform smut nurseries grown at 9 or more of 16 locations throughout the United States during the 5-year period, 1935 to 1939, indicate that only Victoria and Navarro of the smut-resistant varieties listed in Table 3 were completely smut-free in all tests, and that Markton had an average infection of only 0.23% as a result of a trace of infection at two locations.

The introduction of Victoria (C. I. 2401) from Argentina, South America, in 1927, and discovery of its resistance to both smuts and to crown rust, have been reported by Murphy and Stanton (16), Stanton and Murphy (24), and Murphy (11, 13). Because of its outstanding resistance to all races of both smuts and to the prevalent races of crown rust, Victoria, from the standpoint of disease resistance, probably is the most valuable variety of those listed in Table 2. Unfortunately, it is completely susceptible to all races of stem rust, poorly adapted to the principal oat-growing regions of the United States, and further handicapped by late maturity and the presence of heavy, twisted, and geniculate awns.

A history of the development of Markton and the discovery of its high resistance to smut have been reported by Stanton, Stephens, and Gaines (27). Markton is a highly productive variety with yellowish to yellowish-white kernels, but because of its susceptibility to all races of both rusts it is grown successfully in the United States only in the Rocky Mountain and north Pacific Coast states, where neither rust usually is a limiting factor. Because it was the first variety of common oats combining high yield, satisfactory kernel characters, and high resistance to both smuts, Markton has been used extensively as a parent variety in many successful breeding programs for smut resistance. More promising, high-yielding, smut-resistant selections have been developed in the United States from crosses with it than with any other variety.

All available information concerning the origin of the variety Navarro (C. I. 966) has been reported by Stanton (23). Navarro is not known to be susceptible to any races of either smut; however, it has not been subjected to all known races. It is susceptible to both rusts and low in yielding ability, but has proved most valuable in certain breeding programs in the southern states as a source of resistance to the races of smut attacking Fulghum.

The introduction of Bond (C. I. 2733) and discovery of its resistance to crown rust and smut were reported by Stanton and Murphy (24) and Murphy (11). Bond is most valuable as a source of near immunity from most races of crown rust. It has been highly resistant to collections of smut collected from the Corn Belt, but moderately susceptible to many collections of smut from the South, particularly those collected from the varieties Red Rustproof and Fulghum. Bond had an average of 1.12% of infection in the uniform smut nurseries previously referred to with infection observed in one or more of the 5 years at 5 of the 16 locations where the nurseries were

grown. The objectionable characters of Bond are its complete susceptibility to all races of stem rust, the pronounced "sucker mouth" type of base of the primary kernel, and a reddish-yellow grain with a fairly heavy awn. In addition to its high resistance to crown rust and moderate resistance to smut, Bond has a stiff straw of excellent standing ability and produces a plump kernel. It also has fair yielding ability.

Reed (21) demonstrated in 1932 that Black Mesdag was susceptible to the Fulghum race of covered smut. Prior to that date, Black Mesdag served widely as a source of smut resistance. Reed (22) recently showed that Black Mesdag was highly resistant to all of his 29 races of loose smut, but highly susceptible to 3 of his 14 races of covered smut. In the uniform smut nurseries mentioned above, Black Mesdag had an average infection of 3.44% for the 66 station years, with infection recorded at 5 of the 16 locations. Besides its objectionable black grain Black Mesdag is completely susceptible to both rusts and lacks productiveness.

#### VARIETIES RESISTANT TO CROWN RUST

Because Bond and Victoria were resistant to so many more races than any other previously known crown-rust-resistant varieties, their introduction marked the beginning of a new era in breeding for crown-rust resistance in North America.

Bond is highly resistant to 48 of the 51 races of crown rust known to occur in North America, and Victoria is resistant to 47 of these races. Except for the relatively rare race No. 45, to which Bond is susceptible, the other two races (Nos. 33 and 34) attacking Bond and the four races (Nos. 41, 50, 52, and 53) attacking Victoria have been exceedingly rare in all collections identified from the United States, Canada, and Mexico in the 14-year period 1927 to 1940. Race 45 was identified for the first time in 1937 among collections obtained from Texas, Arkansas, Missouri, Florida, Iowa, and Minnesota. Since then, race No. 45 either has not been identified or its prevalence has been limited to one location each year.

Murphy (12, 14) and Murphy, *et al.* (15) have shown that the protection from crown rust afforded by the Bond type of resistance is superior to that obtained from the Victoria type of resistance. Results from these same investigations indicate, however, that the Victoria type is almost as effective and that it should be adequate under most natural field conditions.

Rainbow (C. I. 2345) is susceptible to a number of the common races of crown rust but has shown considerable resistance under field conditions. Besides its moderate resistance to crown rust, Rainbow is resistant to the common races of stem rust and is a high-yielding variety. It is susceptible to both smuts.

Alber (C. I. 2766) and Capa (C. I. 2765), both introduced from South America in 1929 (24), have been used to a limited extent as crown-rust-resistant parents in breeding programs in the United States. Like Rainbow, they are susceptible to a number of the common races of crown rust but have shown considerable resistance under field conditions (11). Both varieties are susceptible to smut and stem

rust. Alber is among the highest-yielding varieties in Louisiana and northern Florida.

Additional varieties, outstandingly resistant to important races of crown rust, are Landhafer aus Uruguay (C. I. 3522), Mutica Ukraina (C. I. 3259), and Trispermia (C. I. 4009). Landhafer aus Uruguay (*A. byzantina*) was used by Straib (28) as a differential variety for identifying races of crown rust in Germany. It was resistant to all 19 races of crown rust collected throughout the United States in 1938, as was Bond, whereas Victoria was resistant to 16 of the races. In nursery tests at Ames, Iowa, in 1938, 1939, and 1940, Landhafer aus Uruguay was lower in resistance than either Bond or Victoria. Landhafer aus Uruguay is susceptible to the smuts and stem rust. Mutica Ukraina is highly resistant to the races of crown rust to which Bond or Victoria are susceptible, but it is completely susceptible to some of the most common races. It is a very later-maturing variety and is highly susceptible to the two smuts and stem rust. Trispermia, a variety introduced from Rumania by the Canadian Department of Agriculture, is reported by Newton, *et al.* (19) as resistant to races Nos. 1, 2, 3, 4, 5, 6, 24, 39, and 45 of crown rust. This variety, belonging to *A. sativa*, was less resistant than Victoria to four of the above nine races but may be of considerable value for breeding purposes. Newton, *et al.*, found Trispermia susceptible to 12 races of stem rust. Preliminary tests at Ames, Iowa, indicate that Trispermia is resistant to loose and covered smut.

#### VARIETIES RESISTANT TO STEM RUST

In 1937, Levine and Smith (9) described the reaction of 27 oat varieties to 10 parasitic races of oat stem rust. Newton, *et al.* (19) recently described the reaction of 78 oat varieties to 11 races of stem rust. Levine and Smith (9) reported the distribution of physiologic races of oat stem rust in the United States during the 15-year period 1921 to 1935. Unpublished results of cooperative investigations by the Minnesota Agricultural Experiment Station and the U. S. Dept. of Agriculture have been made available for the 4-year period 1936 to 1939. These compiled data indicate that races Nos. 1, 2, and 5 comprised 99.2% of the 1,678 isolates identified from the United States during the 19-year period 1921 to 1939, with race No. 2 comprising 70.5%, and race No. 5, 27.2%. Of the 13 known races, Nos. 1, 2, 5, 7, 8, 10, and 12 were identified during the period and, except for the first three, each of the remaining races comprised less than 0.4% of the total distribution.

Richland (C. I. 787) and Iogold (C. I. 2329) are well-known, early-maturing, stiff-strawed, high-yielding varieties, which together comprise about half the annual Iowa oat acreage. Levine and Smith (9) and Newton, *et al.* (19) found Richland and Iogold resistant to the common races Nos. 1, 2, and 5 of stem rust, and also to the rare races Nos. 3, 7, and 12. They were susceptible to races Nos. 4, 6, 8, 10, and 13. Of these latter races only Nos. 8 and 10 have been collected from oats in the United States and their occurrence has been rare. In addition to their outstanding resistance to stem rust, Richland and Iogold are resistant to certain races of smut, Richland being notable

for its resistance to certain of the races attacking Fulghum. Both varieties, however, are highly susceptible to most of the common races of both smuts. They also are completely susceptible to all the races of crown rust. Rainbow has approximately the same reaction as Richland and Iogold to the races of stem rust mentioned above.

Other varieties behaving like Richland, Iogold, and Rainbow in respect to their reaction to the various races of stem rust are Hajira (C. I. 1001) and Rusota (C. I. 2343). Hajira is very similar to Richland in reaction to stem rust and in plant characters. Rusota is a selection from Green Russian of the same origin as Rainbow.

White Tartar (C. I. 551) and Green Mountain (C. I. 1892) are similar morphologically and in their reaction to the various races of stem rust. They possess only moderate resistance to the common races Nos. 1, 2, and 5 but have the advantage of being resistant to races Nos. 8 and 10 which are known to occur occasionally in the United States. White Tartar and Green Mountain are resistant to a few of the more common races of crown rust. Both White Tartar and Green Mountain are susceptible to most of the common races of both smuts.

The origin of Jostrain (Joanette Strain) (C. I. 2660) and a similar variety Sevenothree (C. I. 3251) is described by Bailey (1). These two varieties are resistant to races Nos. 1, 3, 4, and 10 of stem rust and indeterminate in reaction to race No. 5. Because of their complete susceptibility and partial susceptibility to common races Nos. 2 and 5, respectively, their resistance under field conditions has not been satisfactory. It will be noted later, however, that selections resistant or moderately resistant to 12 races of stem rust have been developed from a cross of Hajira Strain  $\times$  Jostrain.

#### DEVELOPMENT OF SUPERIOR OAT VARIETIES RESISTANT TO THE SMUTS AND RUSTS

Successful breeding for disease resistance is directly dependent upon available strains resistant to the various causal agents. The history of breeding for disease resistance in oats in the United States is largely a history of the development or discovery of parental material resistant to the major pathogens. The discovery of the Black Mesdag and Markton resistance to smut, the White Tartar and Kherson (Richland, Iogold, Hajira) resistance to stem rust, and the Victoria and Bond resistance to crown rust and smut, all mark epochs in the progress of oat improvement. It is hoped that as new races of these organisms appear, as they undoubtedly will, sources of resistance to them may be already available or can be obtained readily. Fortunately, the extremes of versatility and pathogenicity for races of the rusts and smuts seem to have become fairly well established, i.e., new races are not likely to be more aggressive and more virulent than some already known, although exceptions may occur.

An attempt has been made to compile a list of improved oat varieties and selections now available in the United States and Canada that possess yielding capacity, quality, etc., combined with adequate resistance to one or more of the major oat pathogens (Table 4).

TABLE 4.—Improved oat varieties and selections resistant to one or more of the major oat diseases.

Variety or selection	C.I. No.	Year of origin	Institution where developed	Resistant to the Smuts	Parent material	Other superior characters
Markton.....	2053	1911	Ore. Exp. Sta.; U.S.D.A.	C.I. No. 357	Sir., D., X Markton	High yield, thin hull
Marleton.....	2378	1919	Are. Exp. Sta.; U.S.D.A.	Markton X Victory	Markton X Victory	High yield, quality, white grain
Marlock.....	2378	1919	Are. Exp. Sta.; U.S.D.A.	Markton X Victory	Markton X Victory	High yield, quality, white grain
Markton X Victory.....	2958	1923	Aberdeen, Idaho, Subst.; U.S.D.A.	Markton X Victory	Markton X Victory	High yield, quality, white grain
Markton X Victory.....	3976	1923	Aberdeen, Idaho, Subst.; U.S.D.A.	Markton X Victory	Markton X Victory	High yield, quality, white grain
Bridger.....	2611	1923	Mont. Exp. Sta.; U.S.D.A.	Markton X Victory	Markton X Victory	High yield, quality, white grain
Marida.....	2571	1923	Idaho Exp. Sta.; U.S.D.A.	Markton X Victory	Markton X Victory	High yield, quality, white grain
Markton X Idamine.....	2574	1923	Aberdeen, Idaho, Subst.; U.S.D.A.	Markton X Victory	Markton X Victory	High yield, quality, white grain
Markton X Idamine.....	2579	1923	Aberdeen, Idaho, Subst.; U.S.D.A.	Markton X Victory	Markton X Victory	High yield, quality, white grain
Markton X Idamine.....	2585	1923	Aberdeen, Idaho, Subst.; U.S.D.A.	Markton X Victory	Markton X Victory	High yield, quality, white grain
Marion.....	3050	1923	Utah Exp. Sta.; U.S.D.A.	Markton X Victory	Markton X Victory	High yield, quality, white grain
Coler, Fulgrain.....	3253	1923	Utah Exp. Sta.; U.S.D.A.	Markton X Victory	Markton X Victory	High yield, quality, med. early
Coler, Fulgrain.....	3257	1923	Coker's Ped. Seed Co., S. C.	Norton No. 20-93 X Navarro	High yield, quality	Cold resist., quality, early
Pulton.....	—	1926	Kans. Exp. Sta.; U.S.D.A.	Fulghum X Markton	Fulghum X Markton	High yield, quality, early
Kansas Sel. 314138.....	—	1926	Kans. Exp. Sta.; U.S.D.A.	Fulghum X Markton	Fulghum X Markton	High yield, quality, early
Kansas Sel. 333293.....	—	1926	Kans. Exp. Sta.; U.S.D.A.	Fulghum X Markton	Fulghum X Markton	High yield, quality, early
State Pride X Markton.....	3491	1928	Wis. Exp. Sta.	State Pride X Markton	State Pride X Markton	Early maturity
O.A.C. 144 X Markton.....	3492	1928	Wis. Exp. Sta.	O.A.C. No. 144 X Markton	O.A.C. No. 144 X Markton	Stiff straw
Richland.....	787	1906	Iowa Exp. Sta.; U.S.D.A.	Resistant to Stem Rust	Richland	High yield, stiff straw, early
Logold.....	2359	1906	Iowa Exp. Sta.; U.S.D.A.	Richland	Richland	High yield, stiff straw, early
Iowa No. 444.....	2351	1907	Iowa Exp. Sta.; U.S.D.A.	Richland	Richland	High yield, stiff straw, early
Anthony.....	2144	1918	Iowa Exp. Sta.; U.S.D.A.	Richland	Richland	High yield, quality
Anthony.....	2144	1918	Iowa Exp. Sta.; U.S.D.A.	Richland	Richland	High yield, quality
Lowa D67.....	2870	1918	Iowa Exp. Sta.; U.S.D.A.	Richland	Richland	High yield, quality
Lowa D67.....	2463	1918	Iowa Exp. Sta.; U.S.D.A.	Richland	Richland	High yield, quality
Hawkeye.....	2464	1918	Iowa Exp. Sta.; U.S.D.A.	Richland	Richland	High yield, quality
Rusoka.....	2343	1922	N. Dakota Exp. Sta.	Richland	Richland	High yield, quality
Morota.....	2344	1922	N. Dakota Exp. Sta.	Richland	Richland	High yield, quality
Rambow.....	2345	1922	N. Dakota Exp. Sta.	Richland	Richland	High yield, quality
Vanguard.....	3478	1926	Dom. Rust. Res. Lab., Winnipeg	Richland	Richland	High yield, quality
Hajira Str. X Jostrain 800.....	4023	1926	Dom. Rust. Res. Lab., Winnipeg	Richland	Richland	High yield, quality
Hajira Str. X Jostrain 818.....	4024	1926	Dom. Rust. Res. Lab., Winnipeg	Richland	Richland	High yield, quality
Hajira Str. X Jostrain 824.....	4025	1926	Dom. Rust. Res. Lab., Winnipeg	Richland	Richland	High yield, quality
Hajira Str. X Jostrain 824.....	4026	1926	Dom. Rust. Res. Lab., Winnipeg	Richland	Richland	High yield, quality





This list is somewhat incomplete because numerous promising selections are now available from many different sources. The more outstanding selections from different crosses at various stations have been listed, wherever known, with preference being given to higher or more inclusive types of disease resistance along with superior yield and quality. The named varieties, with few exceptions, either have been distributed or are being increased for distribution.

#### RESISTANT TO THE SMUTS

It is noteworthy that all of the superior varieties and selections listed in Table 4 as being resistant only to the smuts obtained their resistance from Markton or Navarro. Possibly some selections with the Black Mesdag type of resistance to smut belong in this group; but, as previously mentioned, this type of resistance is not adequate against the increasingly prevalent Fulghum races of covered smut. Because of their resistance also to crown rust, Victoria and Bond do not appear to have been used as a source of smut resistance alone.

Wherever neither rust is a limiting factor these varieties and selections are highly promising. For example, some of the Markton  $\times$  Victory and Markton  $\times$  Idamine selections are very outstanding for yield and quality in the Rocky Mountain region where neither rust ordinarily is of any consequence. Other varieties, such as Huron, Fulton, Coker Fulgrain, etc., are definite contributions to oat improvement for the regions where they are adapted even though the addition of rust resistance is highly desirable. As a group these varieties and selections offer outstanding parental material for sources of high resistance to smut combined with unusual yielding capacity and superior quality.

#### RESISTANT TO STEM RUST

Some of the most important oat varieties grown in the United States and Canada are included in this group, *viz.*, Richland, Iogold, Anthony, Minrus, Rainbow, Morota, Rusota, and Vanguard. The contribution made to oat improvement by the development of these varieties is obvious. The vast acreage devoted to these stem-rust-resistant varieties in the Mississippi Valley doubtless has been an important factor in reducing the frequency and severity of oat stem rust epiphytotics in that region.

The most widely grown varieties of this group are Richland, Iogold, Rainbow, Rusota, Vanguard, and Iowa D67, which are resistant to the prevalent races Nos. 1, 2, and 5 of stem rust and the rare races Nos. 3, 7, and 12; and Anthony and Minrus, which are moderately resistant to races Nos. 1, 2, 5, 8, and 10. The reaction of Morota, Iowa 444, and Hawkeye to stem rust under field conditions indicates that they belong in the group with Richland, while Iowa D69 behaves like Anthony, White Tartar, etc.

From a breeding standpoint the selections developed by J. N. Welsh of the Dominion Rust Research Laboratory are outstanding, particularly the Hajira  $\times$  Joannette selections which have combined resistance to 12 races of stem rust.



## RESISTANT TO THE SMUTS AND STEM RUST

Only 10 varieties and selections are listed in this group, although it is possible others should have been included. The first selection, Minn. D. C. 11-22-220, has the Black Mesdag type of resistance to smut and the White Tartar type of resistance to stem rust, while the remaining varieties and selections have the superior Markton type of smut resistance and the more efficient but less extensive Richland type of resistance to stem rust. Nakota, which is more or less a "special purpose" hull-less type, Hancock, which is outstanding for its stiff straw, and Miomark have been distributed to farmers.

## RESISTANT TO THE SMUTS AND CROWN RUST

Whereas all of the varieties and selections listed as resistant to stem rust and to the smuts and stem rust were spring-sown common oats primarily adapted to the northern portion of the United States and to Canada, those listed under the above heading are primarily fall-sown types or spring-sown red oats adapted to the winter oat section of the South. Crown rust undoubtedly has been the most serious disease of oats in the South and along with winterkilling one of the most important factors limiting oat production in this region. Heavy losses from the smuts have been frequent in the South, particularly where the Fulghum variety has been grown extensively. Stem rust has not been nearly so prevalent or destructive in this region as crown rust, although as resistance is being obtained to crown rust the damage caused by stem rust appears to be increasing or perhaps just becoming more evident. The presence of combined resistance to crown rust and the smuts in Victoria, along with the greater importance of crown rust and the smuts, and the absence of any stem-rust-resistant red-oat or winter-oat types for parental material doubtless explain the initial trend in oat breeding for the South.

The new varieties Fultex, Ranger, and Rustler, now being distributed for production, appear to have the Victoria type of resistance to crown rust and the smuts combined with the superior qualities of Fulghum and Nortex. The development of these varieties and other selections from these same crosses should contribute greatly to oat production in many parts of the South where they may be adapted and where crown rust infection frequently has been a limiting factor.

Latoria, Lega, Lelina, Lenoir, Levic, Coker-Stanton, De Sota, and other selections from the Lee  $\times$  Victoria cross (18) appear to have combined the vigor, winter hardiness, and excellent grain characters of Lee with the crown rust and smut resistance of Victoria. The yields of these selections have been high in Virginia, North Carolina, Georgia, and Arkansas, and they even may be of promise in Oklahoma, Texas, and other parts of the winter oat region. Because fall-sown oats usually escape stem rust infection, except in the more southern area, these crown-rust and smut-resistant Lee  $\times$  Victoria selections may contribute as much to winter oat improvement as the Victoria  $\times$  Richland selections have contributed to oat improvement in the Corn Belt.

Except for lack of resistance to stem rust, which may not be so important in part of the area where these selections are adapted, all the selections in this group are very promising in respect to yield and resistance to crown rust and smut. They meet a definite and immediate need and probably will be superseded only by selections of equal resistance and performance that have the addition of stem rust resistance.

#### RESISTANT TO THE SMUTS, STEM RUST, AND CROWN RUST

Some of the most important of all contributions to oat improvement probably are represented by the recently developed selections listed under the above heading in Table 4. In certain of these selections there have been combined high yielding ability, satisfactory quality, and adequate resistance to the races of both smuts and both rusts of common occurrence in North America.

Marion (C. I. 3247) and the other selections from the cross of Markton  $\times$  Rainbow have been outstanding for yield and grain quality in the Corn Belt and northern Great Plains states (2). These selections have not been tested with all races of both smuts and both rusts, but their parentage and reaction under field conditions would indicate that they are resistant to all the races of both smuts to which Markton is resistant, to races Nos. 1, 2, 3, 5, 7, and 12 of stem rust, and to some of the common and several of the rare races of crown rust. While their resistance to crown rust has not been equal to selections with the Victoria or Bond types of resistance, their resistance has afforded considerable protection as is illustrated by the behavior of a group of these selections in the severe epiphytotic of crown rust experienced in Iowa in 1938 (15), and by the yields in Iowa of Marion in comparison with Boone, Tama, and other new and standard varieties which are presented in Table 5. Marion has been distributed to farmers in Iowa and Illinois.

Boone (C. I. 3305), Tama (C. I. 3502), and Vicland (C. I. 3611) are selections from the cross of Victoria  $\times$  Richland (25, 26) that have been named and distributed. These varieties, along with the other selections from the same cross, are outstanding for yield and resistance to all races of both smuts and the common races of stem rust and crown rust. Of the varieties now available to farmers they probably represent the most nearly ideal combination of high yield and quality with resistance to the four major diseases. Their development appears to be a milestone in the progress of oat improvement.

The selections from Iowa D69  $\times$  Bond and Bond  $\times$  Iowa D67 crosses (17), and the Bond  $\times$  Double Cross A, and Bond  $\times$  Anthony crosses (7) all have the White Tartar type of resistance to stem rust combined with the Bond type of resistance to crown rust and the smuts. As previously mentioned, the Bond type of resistance to the smuts does not give complete protection from some of the Red Rust-proof and Fulghum strains of smut, or from race No. 45 of crown rust which was somewhat prevalent in 1937. Their protection from races Nos. 8 and 10 of stem rust is of great potential importance, however, with a possible increase in prevalence of these races, and the Bond type of resistance to crown rust is superior to the Victoria



type. The selections from the Bond  $\times$  Iogold and Richland  $\times$  Bond crosses are similar to the other Bond-hybrid selections, except that they have the more efficient Richland type of resistance to stem rust but lack resistance to races Nos. 8 and 10. Some of the Iowa D69  $\times$  Bond selections have been unusually high yielding in tests conducted in Iowa.

Some of the selections involving crosses of segregates of Victoria  $\times$  Richland and Markton  $\times$  Rainbow with each other and with Red Rustproof, 1415-8, Morota  $\times$  Bond, Forward (C. I. 2608), etc., are very promising for yielding ability and resistance to all races of the smuts and the common races of both rusts. Those resulting from a combination of Red Rustproof, 1415-8  $\times$  (Victoria  $\times$  Richland 5542-1) are the first red oats adapted to southern conditions with combined resistance to both smuts and both rusts.

#### RESISTANCE TO MINOR OAT DISEASES

The possibility of combining resistance to the more important minor diseases with that already available to the rusts and smuts does not seem insurmountable. Definite varietal resistance has been reported for oat blast (3, 4), halo blight (5), bacterial stripe blight (5), and *Pythium* root necrosis (10). O'Brien and Dennis (20) were unable to find any definite resistance to *Helminthosporium avenae*, although the varieties they tested did vary considerably in susceptibility. Fortunately, this latter disease can be partly controlled by various seed treatments.

With a concentrated effort equal to that utilized during the past decade in breeding for resistance to the major oat diseases, it should be possible to make equivalent progress during the next decade in breeding for resistance to the more important minor diseases whenever these minor diseases are deemed to be of sufficient importance to warrant such a concentration of effort.

#### SUMMARY

The major diseases affecting oats in the United States are loose and covered smuts and crown and stem rusts. Among the minor oat diseases which occasionally cause considerable damage are blast (non-parasitic), *Fusarium* blight, halo blight, stripe blight, *Helminthosporium* leaf blotch, *Pythium* root necrosis, and others.

Estimates reported in the Plant Disease Reporter of the U. S. Dept. of Agriculture, Bureau of Plant Industry, for the period of 1919 to 1938 show an annual loss from smuts amounting to about 3.5%, or approximately 40 million bushels, of the oat crop of the United States. The estimated losses from rusts for this same period are 2% or nearly 23 million bushels for crown rust, and 1.2% or about 14 million bushels for stem rust. Recent statistical studies of the effects of crown and stem rust on oat production indicate that these estimates may be much too low.

For the smuts, Victoria is highly resistant to all known races, Markton and Navarro are highly resistant to all but certain rare

racess, whereas Bond and Black Mesdag have adequate resistance under most field conditions.

For crown rust, Bond and Victoria are highly resistant to all except certain rare and little known races. Rainbow, Alber, and Capa may be classed as only moderately resistant. This resistance, however, affords considerable protection to the crop in many sections.

For stem rust, Richland, Iogold, and Rainbow are highly resistant to all races of apparent economic importance, showing susceptibility only to certain rare races. The old White Tartar oat has furnished adequate resistance under certain field conditions. Jostrain (Joanette strain) can be classed only as moderately resistant.

By crossing within this group of varieties, many new strains have been developed with resistance to the smuts only; to stem rust only; to smuts and stem rust; to smuts and crown rust; and to the smuts, stem rust, and crown rust, in combination with high yield and quality. In winter oats, strains combining disease resistance and winter hardiness also have been evolved. Among the new agronomic smut-resistant varieties are Carleton, Bannock, Marida, Huron, Uton, Fulgrain, and Fulton.

Vanguard is the only comparatively new stem-rust-resistant variety that has been named. Certain selections from a cross of Hajira  $\times$  Joanette are outstanding because of their apparent resistance to all races of stem rust.

Among the recently developed smut- and stem-rust-resistant named varieties are Nakota (hull-less) and Hancock. The number, however, with resistance to smut and crown rust is much larger. A list of these follows: Fultex, Ranger, Rustler, Rangler, Letoria, Lelina, Lenoir, Levic, Lega, De Sota, Coker-Stanton, Victorgrain, and Fulgrain Strain 4.

The newly developed and named varieties with resistance to the smuts, crown rust, and stem rust include Marion, Boone, Tama, and Vicland.

Combining resistance to the more important minor diseases with that already available to the smuts and rusts does not seem impossible. Definite varietal resistance has been reported for oat blast, halo blight, bacterial stripe blight, Pythium necrosis, etc.

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## LABORATORY GERMINATION OF HARD ALFALFA SEED AS A RESULT OF CLIPPING<sup>1</sup>

ANNA M. LUTE<sup>2</sup>

**H**ARD seeds have been the subject of much discussion in connection with enforcement of state seed laws. Label requirements in regard to germination of alfalfa seeds are not uniform in the various states. However, regulations in the majority of the states and under the federal seed act require that the percentage of germination and of hard seed be stated separately on the label. In a few states the sum of the two may be designated as live seed in addition to the separate statements.

The requirement that the percentage of hard seed be stated is based on the fact that their behavior is somewhat different from and more varied than that of the live seeds which take up water promptly.

Designation of hard seed plus seed which germinates promptly as the total live seed is based on the premise that hard seeds if scarified will germinate readily, i. e., hard seeds are live seed.

It is the purpose of this paper to bring out certain pertinent facts regarding the behavior of hard seeds when fresh and after storage.

The term "hard seeds" as used in this paper refers to those alfalfa seeds which do not take up moisture in 6 days when placed between moist blotters in chambers at 20°C.

Much work has been done on the behavior of hard seeds in germination chambers and in the field. The Colorado Seed Laboratory has been carrying on hard-seed studies for more than 20 years and has published one bulletin on the subject.<sup>3</sup> The writer has been unable to find any published work dealing with the following characteristics: (1) whether fresh alfalfa seeds which have taken up no water in blotters at 20°C in 6 days are all live seeds; (2) whether all hard seeds become permeable in storage, resulting in prompt germination, or whether some remain hard over a long period of years; and (3) whether seeds which do remain hard in storage are live seeds.

### MATERIALS AND METHODS

The materials used consisted of five separate sets of alfalfa seed as follows: (1) Fresh seeds containing varying percentages of hard seeds and with fully mature and somewhat immature seeds; (2) old seeds, the original germination and hard seed content of which are known; (3) mature and immature seeds selected from the same bulk; (4) hard seeds taken from blotters in 1921; and (5) seeds treated with heat before storing. All seeds used have had laboratory storage in loosely stoppered vials.

All germination tests were made by placing seeds between folds of moist blotting paper and keeping them at a temperature of 20°C in germination chambers for 6 days. Preliminary and final counts of seeds considered as germi-

<sup>1</sup>Contribution from the Seed Laboratory, Colorado Agricultural Experiment Station, Fort Collins, Colo. Received for publication August 28, 1941.

<sup>2</sup>Seed Analyst.

<sup>3</sup>LUTE, ANNA M. Hard seeds of alfalfa. Colo. Agr. Exp. Sta. Bul. 326. 1928.

nated were made on the third and on the sixth day, respectively. All seeds which had not taken up water at the end of that period were considered to be hard seed.

In order to permit hard seeds to take up water it is necessary to make an opening through the impermeable layer which surrounds the seed, lying just outside of the palisade layer. It has been previously found that a small opening is sufficient to permit intake of water for germination. To make such an opening a very small piece was clipped from each hard seed with a sharp scalpel. Very few of the large number of seeds clipped showed that the embryo had been injured in the process. The clipped seeds were then germinated.

## EXPERIMENTAL RESULTS

### FRESH SEEDS

The hard seeds with which labeling provisions of seed laws are most concerned are seeds which were harvested less than two years previously, since most alfalfa seeds on the market are in that age class. In order to determine whether fresh hard seeds as received in commercial samples are really live, as is sometimes assumed in labeling regulations, a series of 52 samples with varying hard-seed content was studied in the fall of 1938. These seeds were all current samples of the crop of 1937, selected because they were well matured, thus eliminating the possible complication of immature seeds. Upon completion of germination tests all hard seeds were removed from blotters, clipped, and subjected to germination tests. All such seeds germinated promptly as indicated in Table 1. While great care was exercised in clipping seeds, a few embryos apparently were mechanically injured as indicated by broken sprouts.

These 50 samples of fresh commercial alfalfa seeds with hard-seed content varying from 53% to 1% appear to show that fresh hard seeds may very well be called live seed.

### OLD SEEDS

There are fewer hard seeds in old than in recently harvested alfalfa seeds. To determine the percentage of hard seeds present in old seeds as compared to the original content and to find whether old hard seeds are capable of germination, studies were made of seeds which represented bulk lots of old seeds which represented individual plants.

The seeds from bulks studied consisted of 26 lots of alfalfa seeds of the crop of 1925 stored in the seed laboratory until the summer of 1938. Germination tests made at that time showed that seeds which originally had a total of from 97 to 100% germination plus hard seed showed small reductions (up to 24%) in that total, but a much higher germination and correspondingly lower hard-seed content than they did 13 years earlier. The 26 samples were divided into three groups on the basis of the original hard-seed content, as indicated in Table 2.

The first group consisted of 12 samples with a very high hard-seed content, i. e., from 50 to 95%. When retested in 1938 the hard-seed content had fallen, the range being from 10 to 19%. The reduction was not consistently in keeping with the original content, a correlation coefficient of 0.05, which cannot be considered statistically



significant, being obtained. One sample which had 95% hard seed in 1925 had only 19% in 1938, while one sample which had 50% originally had 25% 13 years later. The total germination plus hard seed showed the least reduction, ranging from 2 to 22%.

TABLE 1.—Germination percentage of 50 current commercial samples of mature alfalfa seed of the crop of 1937 tested in October and November, 1938.

Hard seed, %	Germination of clipped hard seed, %	Hard seed, %	Germination of clipped hard seed, %
53	100	11	100*
46	100	10	100
43	100*	10	100
41	100	10	100
36	100	10	100
36	100	9	100*
27	100	7	100
26	100	7	100
23	100*	7	100
22	100	7	100
21	100*	6	100
21	100	6	100
20	100*	6	100
20	100*	6	100
19	100	6	100
19	100	6	100
17	100	5	100
17	100	5	100
16	100	5	100
15	100*	4	100
15	100	4	100
14	100	4	100
14	100	2	100
13	100	1	100
13	100	1	100

\*One seed germinated abnormally, apparently because of injury in clipping.

The second group of eight samples had from 2 to 10% hard seeds in 1925. In this group the hard seed content had not changed materially, being from 2 to 9% in 1938, but there was a noticeable reduction in the total of germination plus hard seeds, this reduction running from 12 to 28%.

A third group of samples, six in number, tested in 1925 had no hard-seed content and a germination of from 53 to 75%. When these lots were again tested in 1938, the germination had decreased, the reduction varying from 11 to 34%.

In the last group the interpretation is simple since the germination percentage was not complicated by the presence of hard seeds at either date. The reduction in live seed was entirely due to death of immediately germinable seeds.

It appeared to be worth while to study the two lots which had hard seed in 1925 and also in 1938 to see whether all seeds remaining hard at the latter date were live seeds. In order to do this all hard seeds remaining in the four replicates (100 seeds each) of each germination test were carefully clipped. In 8 of the 12 samples which had original

high hard-seed content, practically all hard seeds were found to germinate readily after clipping. In the other four lots only 75% responded by germination.

In the eight samples with low hard-seed content the percentage of hard seeds which germinated following clipping was extremely varied, ranging from 22 to 100%. It is to be remembered that the number of clipped seeds from these lots was small and that the percentages do not represent the same degree of accuracy as in the high hard seed percentage samples; they indicate a higher percentage of dead seed among those which were hard.

TABLE 2.—*Germination percentage of 13-year-old hard alfalfa seeds clipped and tested in 1938.*

Germination plus hard seed, %		Hard seed, %		Germination of hard seed, 1938 (clipped), %
1925	1938	1925	1938	
High Hard-seed Content				
100	98	85	32	98
100	96	94	27	100
100	88	74	25	95
100	97	94	27	100
99	91	64	19	100
98	95	50	25	100
97	91	76	49	100
97	89	65	22	97
99	75	95	10	78
99	82	91	25	72
98	74	94	33	74
97	75	95	19	75
Av. 99	88	81	26	91
Low Hard-seed Content				
79	65	5	6	22
76	48	7	5	60
74	61	6	9	84
73	61	5	2	100
71	65	10	7	57
67	40	6	3	83
65	53	2	7	83
55	43	5	2	67
Av. 70	54	6	5	68
No Hard-seed Content				
75	41	0	0	—
69	46	0	0	—
67	54	0	0	—
67	36	0	0	—
66	55	0	0	—
53	27	0	0	—
Av. 66	43	0	0	—

Table 2 shows that change from hard seeds to permeable seeds in different lots is variable, and also that in some lots of old seeds many of the seeds which remain hard are dead.

The seeds which represented individual plants were collected in 1926 and carefully threshed by hand to prevent scarification. All seeds were judged from their appearance to be mature. Tests made soon after harvest showed a hard-seed content varying from 62 to 98% for the individual plants. Tests made of the same lots of seed in 1938 showed that 12 years later the hard-seed content varied from 11 to 75%. The average hard seed for the 25 plants at the time of the original tests was 86%; the average 12 years later was only 31%. Seeds from one plant showed a reduction of only 2%, while another showed 86% reduction.

All seeds from four replicates (100 seeds each) of each lot tested in 1938 which remained hard at the end of 6 days were clipped and returned to moist blotters to germinate. A very high percentage produced prompt, normal sprouts. In most cases failure to germinate normally did not appear to be due to injury in clipping.

Since, so far as it was possible to judge, all lots were mature and all had the same careful hand threshing and the same kind of storage, it would appear that not all hard seeds behave alike. Table 3 gives the details for the 25 lots of seed which were considered.

TABLE 3.—*Germination percentage of 25 lots of hand-threshed alfalfa seed from the 1926 crop, each lot being from an individual plant.*

Hard seed, % in 1926	Hard seed, % in 1938	Germination of clipped hard seed in 1938, %
98	12	100
96	28	94
95	11	78
94	50	94
94	35	91
93	29	83
93	26	89
91	25	89
91	20	86
90	40	95
90	20	98
89	43	93
89	15	100
88	51	99
88	25	100
87	48	97
87	20	96
86	43	95
86	36	80
77	75	92
76	36	92
70	18	97
67	18	79
66	25	100
62	38	91
Av. 86	31	92

Seeds with very high hard-seed content in 1926 (from 62 to 98%) dropped from 11% to 75% of hard seeds in 1938. One sample had practically as many hard seeds in 1938 as in 1926 (75% as compared to 77%), while one sample which had 98% hard seed in 1926 had only 12% in 1938.

When these hard seeds were clipped in 1938 a very high percentage of them germinated. As before, in most cases, failure of clipped seeds to germinate did not appear to be due to injury in clipping.

#### MATURE AND IMMATURE SEEDS

Since previous study indicated that there are some differences between mature and immature seeds, it seemed well to compare the behavior of mature and immature hard seeds from the same bulk.

From the crop of 1938 it was found possible to select eight lots which had both mature and immature seeds present. Careful separations were made with the aid of a hand lens, using color and plumpness as guides. The groups designated as mature were uniform in color and plumpness; those designated as immature varied in color, size, and plumpness.

Germination tests then made showed that at the end of 6 days the hard seed varied from 9 to 92% in the mature seeds and from 12 to 40% in the immature seed. These hard seeds were then clipped and tested for germination. Since in each case the mature and immature seeds had been selected from the same bulk, a direct comparison between behavior of hard mature seed and hard immature seed was possible. In a 6-day germination test 99% of the hard mature seeds germinated readily after clipping. The 1% not counted as germinated appeared from abnormal growth to have been injured in the clipping process. The behavior of clipped immature seeds showed greater variation, as might be expected, from differences in development as indicated by color and plumpness. Their average germination was 84% as compared with the mature seed average of 99%. The variations in individual samples was from 47 to 100%. There was evidence of occasional seeds having suffered damage in the clipping process. The details are given in Table 4. The mature and immature seeds in horizontal rows are from the same bulk.

There were more hard seeds among mature than among immature seeds. A higher percentage of mature than of immature hard seeds were live. The hard mature seeds in these lots were practically all live seeds. Some hard immature seeds were dead.

A second study of mature and immature seeds taken from the same lots was made with seeds which had been hand threshed in 1925 and stored in the seed laboratory until 1938. When tested in 1926, the hard seed content was from 70 to 94%. Careful separations of the 13-year-old seeds were made into mature and immature seeds as was done with the fresh seeds. Germination tests were then made of all lots. All hard seeds remaining at the end of 6 days were clipped and again subjected to germination tests.

Upon germination the hard seed content of the mature lots was found to vary from 10 to 58%, with an average of 35%, while for

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91	20	86
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90	20	98
89	43	93
89	15	100
88	51	99
88	25	100
87	48	97
87	20	96
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86	36	80
77	75	92
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Upon germination the hard seed content of the mature lots was found to vary from 10 to 58%, with an average of 35%, while for

immature seeds it varied from 10 to 65% with an average of 34%. The percentage of clipped mature seeds which germinated varied from 67 to 98%, with an average for the 14 samples of 87%. The percentage of clipped immature seeds which germinated varied from 23 to 94%, with an average of 45%. In 10 of the 14 immature lots more than one-half the seed showed no sign of life in germination blotters after clipping, while no mature sample showed less than 67% germination. These results show the same trend as the fresh seeds of the 1938 crop.

TABLE 4.—*Germination percentage of mature and immature hard alfalfa seed from the 1938 crop tested in November, 1938.*

Mature		Immature	
Hard seed in chamber germination, %	Germination of hard seed, %	Hard seed in chamber, %	Germination of hard seed after clipping, %
92	97	13	77
84	100	36	93
50	100	12	100
50	98	15	47
49	99	40	93
41	100	27	98
37	97	30	78
9	100	19	89
Av. 51	99	24	84

Germination induced by clipping indicates that although seeds may remain hard for 13 years and yet be dead, there are fewer such seeds among the mature than among the immature seeds. Table 5 gives the details for the 14 samples of old seed considered.

#### HARD SEEDS COLLECTED IN 1921

In order to study hard seeds alone, alfalfa seeds which had taken up no water at the end of 6 days in germination tests in 1921 were stored in the seed laboratory for 20 years. These seeds were tested for germination in 1941. The seeds which remained hard at the end of the germination test were clipped and put into moist blotters for germination. These tests showed that many of the old seeds, hard in 1921, had become permeable under laboratory storage conditions. The germination for the 11 samples considered varied from 77 to 86%, with an average of 82%. The seeds remaining hard varied from 6 to 19%, with an average of 13%. A small percentage of the seeds which had become permeable were dead, decaying in 6 days in germination chambers. These dead seeds varied from 2 to 12% with an average of 5%.

The results of clipping the seeds which remained hard at the end of a 6-day test in 1941 show that a few seeds which were still hard were dead. Germination after clipping varied from 78 to 100%, with

TABLE 5.—*Germination percentage of mature and immature hard alfalfa seed hand threshed from the 1925 crop and tested in November, 1938, after 13 years storage in vials.\**

Mature		Immature	
Hard seed, %	Germination of hard seed when clipped, %	Hard seed, %	Germination of hard seed when clipped, %
58	88	39	25
57	67	32	43
56	87	33	56
47	97	65	43
43	87	10	45
40	98	49	51
35	71	25	42
34	98	41	34
32	80	38	60
30	98	30	94
25	67	47	45
18	93	23	30
13	92	37	23
10	91	10	36

\*Germination tests made November, 1938, in chambers at 20°C. Mature and immature in horizontal rows taken from same bulk of seed.

an average of 92%. The remaining seeds decayed at once. Details for these 11 samples are given in Table 6.

TABLE 6.—*Germination percentage of 20-year-old alfalfa seeds which were hard in 1921.*

Germination, %	Hard seed, %	Dead, %	Germination, hard clipped, %
80	15	5	97
82	10	8	100
79	16	5	100
85	12	3	96
82	6	12	78
78	17	5	85
86	11	3	95
77	19	4	92
86	12	2	88
86	12	2	91
86	8	6	88
Av. 82.5		5	92

From these results it may be concluded that many hard seeds became permeable when stored in a room with low humidity and moderate temperatures. Also, the death rate of seeds which remained hard apparently did not vary greatly from those which became permeable.

#### HEAT-TREATED SEEDS

To ascertain whether hard seeds which had been subjected to dry heat before storage respond to germination conditions in the same



way as untreated seeds, work was carried on with seeds which had been exposed to dry heat at temperatures ranging from 35° to 80° C for periods of 1 to 12 hours. Hand-threshed seeds of the crop of 1926 which had been placed in flasks and subjected to dry heat at various temperatures and for various periods of time to determine the possible effect of moderate heat on hard alfalfa seeds were tested in 1925 for hard seeds and stored in the seed laboratory until 1938. The results of germination tests in 1938 indicate that few hard seeds had persisted as such. Those which remained hard in 18 of the 20 samples which had been heated before storage germinated readily after clipping in 1938. Details for the 20 samples from one lot of seed are given in Table 7.

TABLE 7.—*Germination percentage of hand-threshed alfalfa seed treated with dry heat in 1925 and 1926 and tested in 1938.*

Treatment	Hard seed, % in 1925	Hard seed, % in 1926	Germination of clipped hard seed in 1938, %
35°C, 1 hour.....	25	6	100
4 hours.....	23	12	100
7 hours.....	35	15	100
45°C, 3 hours.....	15	5	100
6 hours.....	28	8	100
50°C, 1 hour.....	15	7	100
1½ hours.....	26	11	95
2½ hours.....	20	11	100
55°C, 2½ hours.....	6	1	100
4 hours.....	12	1	100
60°C, 2 hours.....	14	1	100
2½ hours.....	13	1	100
3 hours.....	12	1	100
8 hours.....	12	1	100
65°C, 1 hour.....	10	2	75
2 hours.....	1	1	100
8 hours.....	9	1	100
12 hours.....	15	1	0
75°C, 1 hour.....	10	1	100
80°C, ¼ hour.....	7	1	100
Average.....	15	4	93

Germination of clipped hard seeds 13 years old which were heated the year they were harvested showed a high percentage of live seed even when the hard-seed content was low.

#### CONCLUSIONS

The following brief conclusions appear to be warranted:

1. Mature, fresh, hard alfalfa seeds are live.

2. Many, though not all, immature fresh alfalfa seeds are live.
3. Many hard seeds become permeable in storage in 13 years.
4. A small percentage of hard seeds have become permeable and are dead in 13 years.
5. A small percentage of seeds still hard at the end of 13 years storage are live.
6. A few seeds, hard at the end of 13 years storage, are dead.
7. The percentage of dead hard seeds is greater in immature than in mature seeds.
8. No great difference was found in the death rate of permeable and hard seeds stored for the same length of time.

## NODULATION AND DRY WEIGHT OF GARDEN PEAS AS AFFECTED BY SULFUR AND SULFATES<sup>1</sup>

H. ZANYIN GAW AND P. N. SOONG<sup>2</sup>

SULFUR and sulfates are used as fertilizers, but their effect on leguminous plants is by no means clear. The results reported by previous workers are conflicting. Hart and Tottingham (2),<sup>3</sup> Pitz (5), Powers (6), and others reported that sulfates in general are beneficial to the growth of leguminous plants, especially calcium sulfate which greatly increases root development and nodule production.

Duley (1), Riemer and Tartar (7), Neller (4), and others claimed that sulfur in elementary form exerts a stimulating effect on clover and alfalfa. On the other hand, Swanson and Miller (9) showed that potassium sulfate does not help clover, while Wilson (10) tested some 21 sulfates and found that they generally depress nodule production.

The work reported in this paper deals with nodulation and dry weight of peas as affected by sulfur and sulfates.

### EXPERIMENTAL PROCEDURES

The tests were carried out in pot cultures conducted in the laboratory where the light intensity and temperature were found to be suitable for good growth of the plants. The pots used were ordinary clay flowerpots purchased at the local market. Each pot contained about 1,500 grams of soil. The sand was of a fine quality obtained along the river banks around Kiating. It was washed with dilute hydrochloric acid and then repeatedly with distilled water. Analysis of sulfate was negative.

Besides elementary sulfur, 10 sulfates were tested, namely,  $\text{CuSO}_4$ ,  $\text{K}_2\text{SO}_4$ ,  $\text{ZnSO}_4$ ,  $\text{BaSO}_4$ ,  $\text{FeSO}_4$ ,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{CaSO}_4$ ,  $\text{CrSO}_4$ ,  $\text{MgSO}_4$ , and  $\text{Na}_2\text{SO}_4$ . They were well mixed with the sand before being put in the pots at the final concentrations of 0.01%, 0.05%, and 0.1%.

The garden pea, *Pisum sativum* L., was used as the test plant. The plants, about the time of germination, were inoculated with pure cultures of nodule bacteria, *Rh. leguminosarum* (no. N<sub>4</sub> of our Rhizobia culture collection, originally isolated from garden pea). The bacterial inocula for each pot was 10 cc of 24-hour culture (bacterial number was about 34,000,000 per cc).

The plants were watered with distilled water whenever it was found to be necessary. The moisture content was generally held at about 30%. For nutrient, 50 cc of modified Crone's solution were added to each pot once a week. Calcium sulfate and magnesium sulfate, two normal constituents of Crone's solution were replaced by calcium carbonate and magnesium chloride, respectively.

Four seeds were sown in each pot. Parallel pots were made for each concentration. Two or three experiments were performed.

The growing period was about 8 weeks. At the end of this period, the plants were removed and the number of nodules on each plant was immediately counted.

<sup>1</sup>Contribution from the Physiological Laboratory, National Wu-Han University, Kiating, Szechuen, China. Aided by a grant from the Rockefeller Foundation to the senior author. Received for publication August 18, 1941.

<sup>2</sup>Associate professor of microbiology and Instructor in Biology, respectively.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 103.

The average was taken from the two parallel pots of four plants each for each concentration. The dry weight of the whole plant (roots and top) was obtained after the material was thoroughly air dried when two successive weighings gave constant results.

### RESULTS

From the data obtained in the present study and given in Tables 1 and 2, it will be seen that among the sulfates tested both  $\text{CaSO}_4$  and  $\text{FeSO}_4$  are undoubtedly beneficial to the plants as far as dry weight and nodule production are concerned. The plants grown in these two sulfates as a whole were much taller and were greener than the controls. The root system was well developed with longer and more branched roots bearing large and healthy-looking nodules. This stimulating effect of  $\text{CaSO}_4$  confirms the previous observations of Hart and Tottingham (2), Pitz (5), Powers (6), and many others.  $(\text{NH}_4)_2\text{SO}_4$  appears to increase the dry weight of the plants but nodulation is somewhat depressed.

TABLE 1.—Average nodule number and dry weight of garden peas, two experiments.

Treatment	0.01% conc.		0.05% conc.		0.1% conc.	
	Dry weight in grams	Nodule No.	Dry weight in grams	Nodule No.	Dry weight in grams	Nodule No.
$\text{CuSO}_4$	0.146	30.3	0.138	12.0	0.115	2.3
	0.125	37.6	0.140	5.1	0.125	1.0
$\text{K}_2\text{SO}_4$	0.123	21.2	0.151	54.3	0.169	21.3
	0.155	39.0	0.140	39.2	0.155	38.1
$\text{ZnSO}_4$	0.144	26.3	0.157	29.6	0.153	36.3
	0.158	42.7	0.176	49.7	0.135	52.4
$\text{FeSO}_4$	0.151	31.7	0.205	53.8	0.160	37.5
	0.160	33.9	0.175	42.5	0.180	48.2
$\text{CaSO}_4$	0.167	31.9	0.158	47.0	0.171	50.6
	0.147	40.6	0.186	54.6	0.172	49.3
$(\text{NH}_4)_2\text{SO}_4$	0.176	29.0	0.182	39.2	0.137	21.0
	0.150	28.6	0.161	19.0	0.172	18.6
Control	0.156	33.5	—	—	—	—
	0.159	37.0	—	—	—	—
	0.157	34.9	—	—	—	—

$\text{ZnSO}_4$  and  $\text{Na}_2\text{SO}_4$  increase nodule production but have not much influence on dry weight. The effect of  $\text{Na}_2\text{SO}_4$  is in line with the observation of Pitz (5) who reported that when  $\text{Na}_2\text{SO}_4$  is applied alone, the plants grow better and bear more nodules. The other sulfates tested in the present study either depressed or did not seem to have much effect on nodulation and dry weight. Among them are  $\text{K}_2\text{SO}_4$  and  $\text{CrSO}_4$  whose action of depression on leguminous plants,

according to Wilson (10), is offset by the metallic ions of K and Cr, but the present data did not bear this out, particularly with respect to  $\text{CrSO}_4$  which depressed both dry weight and nodulation very markedly.

TABLE 2.—Average nodule number and dry weight of garden peas, three experiments.

Treatment	0.01% conc.		0.05% conc.		0.1% conc.	
	Dry weight in grams	Nodule No.	Dry weight in grams	Nodule No.	Dry weight in grams	Nodule No.
S	0.150	21.7	0.157	27.2	0.120	19.7
	0.172	24.0	0.155	21.7	0.137	20.4
	—	14.0	0.137	32.5	0.127	21.2
$\text{Na}_2\text{SO}_4$	0.151	19.2	0.176	16.3	0.143	12.7
	0.167	26.0	0.135	18.5	0.146	25.7
	0.136	24.7	0.127	—	0.131	23.2
$\text{BaSO}_4$	0.130	16.3	0.142	16.5	0.112	19.5
	0.160	22.2	0.116	21.5	0.140	17.7
	0.137	9.5	0.110	31.3	0.137	18.1
$\text{CrSO}_4$	0.106	8.5	0.146	13.6	0.137	10.5
	0.106	19.5	0.133	9.0	0.120	8.0
	0.110	22.0	0.130	11.0	0.112	12.5
$\text{MgSO}_4$	0.142	10.2	0.153	17.0	0.121	18.7
	0.132	18.5	0.130	22.0	0.180	12.7
	0.125	—	0.120	17.4	0.139	11.5
Control	0.187	17.5	—	—	—	—
	0.173	18.7	—	—	—	—
	0.190	18.1	—	—	—	—

The effect of  $\text{CuSO}_4$  was most remarkable. Dry weight and nodule production were greatly reduced. The plants were smaller and were abnormal in appearance, especially the pale green color of the leaves, indicating little or no nitrogen. The root system was quite characteristic, being unusually short and very poorly developed and not only bore few or no nodules but also had very few branched roots.

As for sulfur, the data show that it does stimulate nodule production, although the dry weight of the plants seems to be depressed. This increased nodulation is in confirmation of findings of Duley (1), Pitz (5), and Miller (3).

The effect of sulfur and sulfates on leguminous plants may be explained on the basis of two factors, *viz.*, they act as fertilizers supplying the sulfur needed for plant growth, and they act on nodule bacteria influencing nitrogen fixation. Of these two factors, the second seems to be more important. In the case of  $\text{CaSO}_4$ , for instance, it has been reported by various workers Pitz (5), Miller (3), and others that this sulfate increases the number of nodule bacteria as much as 100%. This leads them to believe that the beneficial effect of plants

grown on the soil where  $\text{CaSO}_4$  had been added is due to the stimulation of nodule bacteria by the sulfate, causing greater nitrogen fixation. As a matter of fact, Singh (8) reported an increase of nitrogen fixation as a result of  $\text{CaSO}_4$  application. On the other hand, sulfates like  $\text{CuSO}_4$  may have a detrimental influence on nodule bacteria, causing inefficient nitrogen fixation and thus affecting the growth of the plants. Wilson (10) reported the toxic effect of  $\text{HgSO}_4$  which kills the nodule bacteria.  $\text{CuSO}_4$  in all probability has something of the same sort of action on nodule bacteria.

### SUMMARY

1. The work described here deals with nodulation and with the dry weight of the garden pea as affected by sulfur and 10 sulfates.
2. It was found that  $\text{CaSO}_4$  and  $\text{FeSO}_4$  increased nodule production and dry weight;  $(\text{NH}_4)_2\text{SO}_4$  increased dry weight but not nodulation;  $\text{Na}_2\text{SO}_4$  and  $\text{ZnSO}_4$  increased nodulation but did not affect dry weight.  $\text{CuSO}_4$ ,  $\text{CrSO}_4$ ,  $\text{K}_2\text{SO}_4$ , etc., depressed nodule production and dry weight.
3. Sulfur in elementary form stimulated nodule production but slightly depressed dry weight.

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## NOTE

## DETERIORATION OF BUR CLOVER SEED UNDER IRRIGATED CONDITIONS

BUR clover, because of its seeding habits, is an excellent winter cover crop when adapted to the particular soil conditions. Normally, it grows best in a well-drained soil. Excellent volunteer stands may be obtained for several years after a crop is permitted to produce considerable seed before being turned under.

In the production of rice in Arkansas it has been found that green manure crops of legumes produce larger yields of rice. The problem involved, however, is to produce economically sufficient green manure to be effective. Bur clover would be an economic source of organic matter if the anaerobic conditions produced by the irrigation of the rice did not decrease too much the longevity of the bur clover seed in relation to its volunteer habits when used as a green manure crop.

In order to test the effect of submerged conditions, seeds of southern bur clover, both hulled and in the bur, and hulled Button bur clover were thoroughly mixed through soil and placed in quart jars and in 2-gallon earthenware jars. The soils were flooded with tap water and maintained in that condition in the greenhouse for the duration of the experiment. At successive monthly intervals for 7 months, a longer period of submergence than would occur in the production of rice, the soil in jars selected at random was washed through a screen which would retain all of the seed and the material left on the screen air dried. The seeds were separated and counted and subjected to a germination test. In the case of the burs the number of burs were first counted and the seed separated from the burs by hand before being subjected to the test. The seeds were also separated from five samples of 100 burs each which were not irrigated to determine the number and viability of the seeds when the experiment was started.

It should be mentioned that the average daily temperature in the greenhouse in all probability was higher than would be encountered under field conditions, although efforts were made to maintain the temperature between 70° and 80° F. This would, of course, tend to increase the rate of any decomposition which might take place, but would not nullify the general conclusions which may be made from the results. Typical results are given in Table 1.

Since the percentage of germination when tested by the standard method was so small, the seeds not germinated after a stated period were given a hot water treatment and placed in the germinator for an additional period. This, according to Casey,<sup>1</sup> gives a reliable index to the viability of the seed.

The results show that deterioration took place quite rapidly. This is especially true of the hulled seed of both southern and Button bur. After only one month's submergence less than 10% of the seed were still in a viable form. While no specific relationship can be noticed

<sup>1</sup>CASEY, JOHN E. Boiling water treatment an aid in germinating bur clover seed. Proc. Assoc. Off. Seed Analysts of North Amer., 31st Ann. Meet., 114. 1939.

between length of submergence, it appears safe to conclude that a survival of less than 10% of the original seeds against the decomposition processes would make it difficult to get a good stand the next fall. Moreover, the chances for securing a good stand would be made less promising because of the fact that most of the seeds were hard seeds. This means that in all probability only a small percentage would germinate each year and the stand would be too thin to be of value as a green manure crop.

TABLE 1.—*Effect of submergence treatment upon the number of bur clover seed in burs remaining in the soil.*

Time in months	Number of burs recovered	Number of seed in burs	Germination				Total number germinated	Viable seeds recovered, %*
			Standard method		Hot water treatment			
			Number treated	Number germinated	Number treated	Number germinated		
In Quart Jars								
1	95	212	212	26	185	153	179	54.6
2	94	155	155	20	131	124	144	46.1
3	100	100	100	1	99	81	82	24.5
4	91	85	85	2	83	75	77	23.5
5	86	70	70	1	69	61	62	18.4
6	93	58	58	0	58	49	49	14.9
7	89	37	37	0	37	25	25	7.6
In 2-gallon Jars								
1	96	325	325	5	320	264	269	82.1
2	96	233	233	73	158	150	223	68.1
3	95	213	213	13	198	174	187	57.0
4	93	142	142	8	134	118	126	38.4
5	98	134	134	4	130	119	123	37.2
6	92	119	119	10	109	82	92	28.1
7	89	94	94	5	89	71	76	23.2

\*The average number of seeds in five samples of 100 burs each was 328 of which 25 germinated by the standard method; 244 additional germinated with hot water treatment, a total of 269 or 89.3% viable seeds before submergence or irrigation was started.

The deterioration of the seed in the burs was not as rapid as that of the hulled seed. However, even in this case, the number of viable seed was reduced to around 50% within a period of 2 months after submergence or irrigation. At the end of 5 months only a small percentage of the seed was still viable. Moreover, the results show that most of the seed remaining after 2 months submergence were hard seed since only a few of the seeds germinated without special hot water treatment.

The results appear to be conclusive that the seed of bur clover will deteriorate if they remain in the soil under water-logged conditions such as encountered in rice production. It is evident, therefore, that



the volunteering characteristic of bur clover is not adapted to the conditions encountered in rice farming and if used as a cover crop would have to be seeded every year.—R. P. BARTHOLOMEW and L. C. CARTER, *Arkansas Agricultural Experiment Station*, and JOHN E. CASEY, *State Seed Laboratory, Fayetteville, Ark.*

## AGRONOMIC AFFAIRS

### SOIL SCIENCE SOCIETY OF FLORIDA

THE fourth annual meeting of the Soil Science Society of Florida was held in Gainesville, December 5 and 6, 1941, at which time 14 papers were presented. Soil research, with special emphasis on Florida agriculture, plant food, soil and water conservation and Inter-American agricultural education and research were discussed.

Mr. C. B. Manifold, Assistant Chief of the Soil Conservation Service, Washington, D. C., discussed tropical soils suitable for rubber culture. Other speakers on the program included Mr. Harold Mowry, Assistant Director of the Florida Agricultural Experiment Station, Dr. A. F. Camp, Horticulturist in charge of the Citrus Experiment Station and Dr. J. R. Neller, Biochemist, in charge of the Everglades Experiment Station.

Doctor Neller was elected President for the year 1942 and Mr. M. J. Mossbarger of Miami was elected Vice President.

### THE NATIONAL SCIENCE FUND

"PHILANTHROPY in Science" is the title of a leaflet issued by the National Science Fund of the National Academy of Sciences from headquarters at 515 Madison Avenue, New York City. The leaflet explains the purpose of the Fund and lists the officers and directors.

In a recent communication to the Secretary of the Society, Dr. William J. Robbins, Chairman of the Board of Directors of the Fund, makes the following comment:

"The National Science Fund was created by the National Academy of Sciences last spring as a means of helping to meet an increasingly urgent need for additional funds to support fundamental scientific research. A three-year study, conducted by a committee of Academy members headed by Dr. Albert F. Blakeslee, retiring President of the American Association for the Advancement of Science, showed clearly that since the early 1930's, financial support of research has been decreasing, and that an authoritative advisory body was needed to assist public-spirited people who wish to make effective gifts to science.

"Under the direction of a board of qualified scientists and distinguished laymen, the National Science Fund is now prepared to administer large or small gifts and to provide a center to which any person or group may go for competent and impartial guidance in making a fruitful investment in science. The Fund plans to work

through existing agencies rather than carry on research of its own.

"The successful development of the Fund over a period of years will depend first of all upon the understanding and wholehearted support of scientists themselves. They have the primary interest in its establishment, its progress, and in the aid which the Fund may ultimately extend to them in their own research work. However, to all individuals and groups concerned with problems of human welfare and the nation's progress, maintenance of scientific advance is also of highest importance."

More detailed information regarding the Fund and its administration may be obtained by writing to Dr. Robbins.

#### STUDENT SECTION ESSAY CONTEST FOR 1942

ACCORDING to an announcement from the Committee on Student Sections of the Society, students presenting the best papers in the student essay contest for 1942 will receive awards as follows: The first three winners receive expense money to enable them to attend the International Grain and Hay Show in Chicago. The total allotment for the three will not exceed \$150.00. The amounts granted will vary with the distance the winners are from Chicago. For example, if a Wisconsin student won first, and an Oregon student second, it would be desirable to grant a larger amount to the Oregon student, as his expenses would be greater. The committee reserves the right to adjust this as conditions warrant. In addition, the three high men will receive appropriate medals and a year's subscription to the Journal of the American Society of Agronomy. The winners of the fourth, fifth, sixth, and seventh places will receive cash awards of \$20, \$15, \$10, and \$5, respectively.

All essays must be prepared by undergraduate students. Students graduating during the course of the 1941-42 school year or those graduating during the summer school of 1942 are eligible providing their papers are submitted before graduation. A certification of eligibility to qualify as an undergraduate, signed by the Dean of the College, must accompany each paper.

Papers should be typed, double spaced, and not less than 3,000 or more than 3,500 words in length. *Abstracts of not more than 500 words must accompany each paper.* Abstracts should be prepared carefully as it is planned to publish the best. Failure to submit an abstract will disqualify the paper.

The title for the essay shall be: "Plants as Indicators of Soil Deficiencies and Crop Adaptation." Subject may be treated for any section or all sections of the United States and from any angle. Ecological phases as lack of drainage, soil acidity, xerophytic and similar conditions may be treated. Essays must not be reviews of the book "Hunger Signs in Crops."

All papers are to be submitted in duplicate and if it is desired that the essay be returned, postage should be enclosed.

The Committee suggests that where several papers are entered from a given institution, the local representatives of the Society review the essays and submit only the best articles. This will save

work for the committee and reduce mailing expenses. Usually not more than five papers should be submitted from one institution for final review by the committee. The winners of the contest will be announced at the meeting of the American Society of Agronomy in the fall of 1942. Results will be published in the December issue of the JOURNAL.

Essays must be in the hands of the Chairman of the Committee, H. K. Wilson, University Farm, St. Paul, Minnesota, not later than August 1, 1942. Agronomists are urged to encourage student participation in this contest.

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### RETENTION OF VITAMINS IN FLOUR AND BREAD<sup>1</sup>

L. H. NEWMAN<sup>2</sup>

AS a result of exacting scientific investigations, dietary surveys, and clinical observations, nutritionists seem to be pretty well agreed that, generally speaking, our diet might be improved upon and our need for certain drug store products lessened if the food we eat were to contain a more liberal supply of the vitamin B complex and especially B<sub>1</sub> or thiamin chloride. Since the wheat kernel is naturally rich in the B complex and since the products of wheat flour constitute the most commonly consumed foods of the people, nutritionists conclude that the simplest and probably the most economical and effective means of increasing our daily intake of these vitamins is through the medium of bread.

While we are told that eight members of the vitamin B complex or group have been isolated, *thiamin* or vitamin B<sub>1</sub> is one of the members which has been found to be present in insufficient quantities in our ordinary diet.

The vitamin B<sub>1</sub> content of foods is measured in International Units, or i.u. Three micrograms (1 million to the gram) of B<sub>1</sub> equal 1 i.u. Whole wheat flour contains ordinarily well over 500 International Units per pound. A rather extensive survey made in Canada of what we know as "Second Patent" flours showed that the B<sub>1</sub> content ranged all the way from 115 to 194, the average being about 160 i.u. per pound. If a larger percentage of our people ate whole wheat bread, the problem obviously would be much less acute, but according to surveys made it would appear that from 85% to 90% of the bread consumed in Canada is white.

The B<sub>1</sub> content of white bread may be increased in any one of three main ways, *viz.*, by the use of high potency yeast, by the addition of synthetic B<sub>1</sub> or thiamin, or by the use of flour in which more of the B<sub>1</sub> has been retained by a process of milling.

In Great Britain, the Food Ministry, recognizing the importance of having an adequate supply of this important "nerve tonic" in the daily food of the people, has decreed that all flour used in that

<sup>1</sup>Contribution from the Cereal Division, Central Experimental Farm, Ottawa, Canada. Presented before the general session of the American Society of Agronomy at the 34th annual meeting held in Washington, D. C., November 13, 1941.

<sup>2</sup>Dominion Cerealists.

country must have added to it at time of milling 0.2 gram of thiamin per English sack of 280 pounds. This same idea has swept across the United States where not only is the addition of thiamin advocated but where certain other important elements are also recommended, at least as a temporary expedient.

In Canada, the medical fraternity is not in favor of the addition of synthetics but prefers that our vitamins be obtained entirely from the natural wheat berry. Various reasons for this point of view have been submitted. McHenry<sup>3</sup> points out that since the various members of the vitamin B complex usually occur in the same foods, it may be assumed that people suffering from an insufficient amount of one vitamin are suffering from a lack of all the members of the same group. In speaking on this subject at a conference held in Ottawa on July 30, Doctor McHenry referred to experiments he had been conducting with individual members of the complex. He stated that the use of six members will produce results up to a point and that the addition of the seventh seems to co-ordinate the efforts of all of them to produce a more desirable effect. It will not solve the problem to add any two or three of them or to augment any two or three of them, he pointed out. The addition of the whole complex synthetically would not be practical from a cost standpoint and he felt that the retention of the complex as found in wheat was absolutely necessary. Man was intended to get the whole group in his diet and an imbalance, he believed, could be definitely harmful.

Dr. F. F. Tisdall of the Department of Paediatrics, University of Toronto, who is also Chairman of the Committee on Nutrition of the Canadian Medical Association, spoke strongly on the same occasion in favor of the principle of seeking a higher vitamin content in our white bread through the utilizing of the natural vitamins in the wheat. He expressed the opinion that there is no other single measure that will so improve the health of the nation as will the retention in our white flour of the maximum natural vitamin content of the wheat berry.

Doctor Thomson, Department of Nutrition, McGill University, Montreal, when discussing the same problem on the occasion of the above conference, stated that in speaking for the nutritionists he was quite confident that they were not wedded to any particular process of milling so long as the restoration of vitamins is accomplished. He stated that they did disapprove of partial restoration at added cost.

Dr. C. H. Best, successor to Sir Frederick Banting, Banting Institute, Toronto, speaking on the same occasion, stated that he also would like to emphasize that the provision of B<sub>1</sub> from natural sources has great advantages over the substitution of synthetic material. He felt that Canadian millers would be performing an extremely important service if they would make every effort to provide a white flour rich in vitamin B<sub>1</sub> such as that which has been described during the discussion.

In view of the attitude of the medical people and nutritionists in Canada on this question, the chief problem involved obviously is a

<sup>3</sup>McHENRY, E. W. Observations on the nutritive value of bread. Can. Pub. Health Jour., 31:428. 1940.

milling one. We must concern ourselves with the problem of having our wheat milled so that more of the B<sub>1</sub> content of the wheat berry is retained in the flour. As to how this might be accomplished without unduly upsetting present milling practices is a problem with which the Cereal Division, in cooperation with the Department of Paediatrics, Toronto University, and the Canadian millers has been grappling during the past 13 months. In this paper a brief statement is submitted on the progress made to date. The milling work was conducted at Ottawa on a Buhler experimental mill under the supervision of Mr. A. G. O. Whiteside who is in charge of flour testing investigations, while all assays for B<sub>1</sub> were made by the Department of Paediatrics, Toronto, under the direction of Dr. F. F. Tisdall.

#### THIAMIN CONTENT OF CANADIAN SPRING WHEAT

In connection with our investigations, the thiamin content of Canadian spring Wheat was first investigated. Typical mill mixtures were obtained from various mills when the former were ready for grinding. The range of B<sub>1</sub> content of these wheats ran from 537 to 735 i.u. of B<sub>1</sub> per pound. The majority however, were very close to 650 i.u. per pound.

Further information on this point was obtained from samples collected direct from export cargoes belonging to the various northern grades at Vancouver and Atlantic ports. These samples were collected by the Board of Grain Commissioners, Winnipeg, each sample representing many thousands of bushels. The assays made on this particular material to date show a rather remarkable uniformity in the B<sub>1</sub> content of these grades. The Garnet grades assayed to date all show a lower B<sub>1</sub> content than do the northern grades from which Garnet is debarred. This may possibly be due to the variety itself or to the district from which it came. Garnet is grown in Canada now almost exclusively in the northern areas because of its early ripening properties.

In these far northern districts the protein content of our wheat, especially of Garnet, tends to run appreciably lower than in other districts.

The problem of the effect of environment on the thiamin content as well as the difference between varieties in each district in this respect have also been investigated. Six varieties, *viz.*, Garnet, Red Bobs, Reward, Marquis, Regent, and Thatcher, were collected from five Dominion experimental stations in western Canada and assayed for B<sub>1</sub> content. This study revealed that Garnet was the lowest at all points, never reaching the 600 mark, while Regent was definitely the highest followed by one of its parents, Reward.

A further collection of six standard varieties was made from 158 widely scattered points throughout the three prairie provinces. The varieties investigated in this case were Apex, Marquis, Regent, Renown, Reward, and Thatcher. The B<sub>1</sub> assays of these samples placed Regent and its full sister Renown at the top with 820 i.u. per pound of wheat. Reward, one of the parents of these two varieties, came second with 806 i.u. per pound. Thatcher gave 700 i.u. per

pound, while Apex and Marquis followed fairly close with 670 and 645 i.u. per pound, respectively. All the samples in this test were of high weight per measured bushel.

### THIAMIN CONTENT OF CANADIAN FLOUR

Following the above study of the thiamin content of whole wheat, we next attempted to locate just where the thiamin is found in the wheat berry. For this study, a sample of Manitoba No. 1 northern spring wheat was used. The first study was made of the germ itself, the pure germ being extracted from the kernel by hand in sufficient quantity to enable an assay to be made. This showed the pure germ to contain 3,300 i.u. of B<sub>1</sub> per pound. Other kernels were then cut crosswise into approximately three equal parts by weight and each part was analysed separately. The assays on these three sections showed that the germ end with the germ included carried 1,700 i.u. of B<sub>1</sub> per pound, while the central section carried only 158 i.u. per pound and the other third of the kernel 222 i.u. per pound.

In another study in which the kernels were cut transversely into two equal parts by weight after the germ had been removed, it was shown that the germ end, even with the germ removed, carried infinitely more thiamin than did the other half of the kernel. The actual assays showed that the germ end with the germ removed carried 915 i.u. per pound, whereas the brush end gave only 252 i.u. per pound. This led us to conclude that the cells immediately surrounding the germ are extremely high in thiamin content. This fact conceivably may prove important from a milling standpoint.

Coming back for a moment to a consideration of the germ itself. While the claims made for the high vitamin content of this fraction apparently are well founded, yet after all, the germ in Canadian wheat, according to our studies, occupies only about 1.5% of the wheat kernel and actually carries only about 13% of the B<sub>1</sub> contained in the whole kernel. Failure to recognize this fact has led a good many people to draw wrong conclusions regarding the contributions made by the germ even though the entire germ were recoverable by any known process of milling. In this connection let us examine the results obtained from one of our initial milling experiments. In this case, we made separate millings of 1,000 grams each of Regent wheat, the milling being done on our experimental Buhler mill at Ottawa. Eight separations were made, a B<sub>1</sub> determination being made on each separation. The results of these assays, as compiled by White-side, appear in Table 1.

An examination of this table will show that the bran separation contributes about two-thirds of the total vitamin B<sub>1</sub> content of the kernel and that the bran, together with the shorts and feeds, actually account for approximately six-sevenths of the thiamin content of the wheat berry. The remaining six streams shown in the table together constitute what we commonly call a straight grade flour.

In Table 2 a picture is presented of how each of the above six flour streams contribute to the B<sub>1</sub> content of the straight grade flour.

TABLE 1.—Assays of  $B_1$  in eight separations of Regent wheat.

Product	$B_1$ content, i. u. per lb.	Percentage of total products	Calculated $B_1$ , i. u. per lb. of wheat
Bran.....	2,080	24.2	502.9
Shorts and feeds.....	2,180	5.2	112.9
1st break flour.....	64.5	4.7	3.0
2nd break flour.....	121	8.5	10.2
3rd break flour.....	306	3.4	10.3
1st reduction flour.....	138	41.0	56.6
2nd reduction flour.....	450	8.6	38.8
3rd reduction flour.....	658	4.5	29.7
		100.1	764.4

It will be seen from Table 2 that the total  $B_1$  content of the six streams which make up our straight grade flour in this particular case yielded a calculated 210.3 i.u. of  $B_1$  per pound of flour. Obviously, if this flour is to be brought up to the required standard, an appreciable quantity of  $B_1$  will have to be extracted one way or another from the feed streams.

In order to obtain further information as to what the various flour streams might be able to contribute, samples were obtained from 29 streams produced by one of our large Canadian mills, each stream being assayed for  $B_1$  content. In this milling 80% of the flour produced came from 11 different separations which had a range in  $B_1$  content of from 61 to 244 i.u. of  $B_1$  per pound of flour. The fifth break, which was one of the highest in  $B_1$  content carried 373 units of  $B_1$  per pound, but this represented only 0.5% of the total flour produced. From another large Canadian mill 27 different flour streams, together with bran, shorts, and feed middlings, were collected and analysed for  $B_1$  content. The combined  $B_1$  content of all these products amounted to 650 i.u. per pound. Of this amount the different flours contributed 101 i.u., or 15.5%, the bran 194 i.u. or 29.9%, the shorts 293 i.u. or 45.1%, and the feed middlings 61 i.u. or 9.5%. The  $B_1$  content of the different products was as follows: Total flour, 138 i.u. per pound; bran, 1,440 i.u. per pound; shorts, 2,710 i.u. per pound; and feed middlings, 2,280 i.u. per pound.

TABLE 2.— $B_1$  content of the flour streams of straight grade flour.

Flour designation	$B_1$ content, i. u. per lb.	Percentage of straight grade flour contributed by each	Calculated $B_1$ in i. u. per lb. of straight grade flour
1st break flour.....	64.5	6.6	4.3
2nd break flour.....	121	12.0	14.5
3rd break flour.....	306	4.8	14.5
1st reduction flour.....	138	58.1	80.1
2nd reduction flour.....	450	12.2	54.9
3rd reduction flour.....	658	6.4	42.0
Total.....		100.1	210.3



The first eight middlings flours, representing the most refined flour streams and amounting to 77% of the total flour, contributed only 70 i.u. of B<sub>1</sub> to the pound of the total flour. If all flours containing over 200 i.u. per pound, except the low grade and brain and shorts dust flour, were combined, the resulting flour would amount to about 17.3% of the total flour and would contain 323 i.u. per pound. While it may be possible to segregate high vitamin B<sub>1</sub> flour streams in a large mill and use these in stepping up the B<sub>1</sub> content of the final flour, the quantity of such flour thus obtained would represent only a small proportion of the total flour produced. It would appear, therefore, that there will have to be some other change in the ordinary milling procedure if large quantities of high vitamin B flour are to be produced. It has been in this particular field that our Department has been groping since we began our studies, the results of which I desire to present briefly in this paper.

#### MILLING INVESTIGATIONS

When we began operations, we learned that Doctor Tisdall had been instrumental in initiating certain milling investigations in connection with a small commercial mill located at Chatham in western Ontario. As a matter of fact this mill under his general guidance and inspiration had progressed to the point where it was producing a high vitamin flour to which had been given the name "Puros". This flour, in turn, was being used by a large bakery concern in Toronto to produce a high B<sub>1</sub> loaf called "Melior". This bread unfortunately was not white but rather a greyish color and, while its use is fairly extensive, it falls outside the white bread class.

While Puros flour was not the answer, yet the method by which it was produced suggested a line of attack which it was hoped might give us the high vitamin white loaf we were seeking. We obtained a quantity of Puros which assayed 525 i.u. per pound and rebolted it through a 10XX silk flour sieve. This was done to remove the specky material which gave the flour the dark color and which resulted from the method by which it was milled. This method called for a moisture content of 13.0% in the case of the wheat going to the rolls and an extraction of from 82% to 83%. The rebolted flour, even after about 10% of this dark specky material had been removed, assayed 420 i.u. of thiamin per pound, while the bread baked from it approached a white loaf. This led the company to decide to see what they could do towards producing a flour which would produce a loaf entitled to be classed as a white loaf and still carry a relatively high B<sub>1</sub> content. The flour they finally developed was given the name "Puros-B", while the loaf which came to be made commercially was given the brand name "Energeat".

In order to obtain some idea as to the public acceptance of this type of loaf, our Department obtained a quantity of Puros-B flour and had it baked commercially by one of the large Ottawa bakeries. This particular flour contained 365 i.u. of B<sub>1</sub> per pound. A second lot, which was a little darker in color and which carried 470 i.u. per pound, was also obtained and baked by the same commercial concern. A

loaf of each lot in plain wrappers was then distributed to 100 homes in Ottawa along with a questionnaire calling for certain opinions as to the acceptability of the color, texture, flavor, etc., of each loaf. Eighty-five replies were received. Eighty-three of these indicated that the lighter colored loaf carrying 365 i.u. of B<sub>1</sub> per pound of flour was entirely acceptable in every respect. Some even stated that they would be very glad indeed to be able to get a loaf of that particular type irrespective of any additional vitamins it might carry. Seventy-five of those receiving the darker colored bread stated that even this color was not objectionable. A number of the latter group commented particularly on the superior flavor of the bread. From these reports, while admittedly subject to criticism, we concluded that in spite of what some would have us believe, the average bread consumer is not definitely wedded to a chalk-white loaf. As a matter of fact, there are a good many reasons to believe that many people would prefer bread which was not quite so white as a good deal of the bread now on our Canadian market. On the other hand, there does not seem to be much evidence that any of our so-called brown or whole wheat loaves are gaining appreciably in popularity.

The experiences of the Chatham company in the milling of wheat at a lower moisture level than that commonly used and of our own work in the rebolting of the flour produced by them suggested that there might be a certain point respecting moisture content of the wheat going to the rolls and also the percentage extraction where best results might be expected. With this in mind, we conducted a series of millings on our experimental Buhler mill in which the moisture levels of the wheat were varied as were the flour extraction percentages. In these early trials, all the flour was passed through a 10XX flour silk sieve in order to remove the dark colored particles which otherwise would darken the flour. The first sample milled was a Manitoba No. 1 northern wheat carrying a natural moisture content of 10.8%, and a thiamin content of 655 i.u. per pound. This wheat was tempered to two moisture levels, *viz.*, 12.5% and 15.5%, the latter being the moisture at which wheat commonly is milled commercially in Canada.

In Table 3 there is shown the influence on the B<sub>1</sub> content as well as on the ash of tempering wheat to two different moisture levels.

Subsequent millings at different moisture contents and at different extractions again showed quite definitely that ash and thiamin content increased as extraction increased. It was also shown that where the extraction was not increased but the wheat milled at a lower than normal moisture content, *viz.*, around 13.0% or 13.5%, that a fairly satisfactory thiamin content might be expected as might a flour of a reasonable degree of whiteness. It was shown further that even where milling is conducted at the 15.5% level but at a higher extraction than normally, thiamin content is increased appreciably.

The extraction in Canada commonly practiced today is about 75%. If this could be increased to around 78% and the moisture content of the wheat going to the rolls reduced from 15.5% to 13.5% or even 14%, or if certain alternative adjustments could be made, there

should be little difficulty in producing a reasonably white flour carrying around 365 i.u. of B<sub>1</sub> per pound. With this in mind, it has been recommended in Canada that the minimum thiamin content of our so-called Canada Approved High Vitamin White Flour be set at 360 i.u. per pound. It is hoped however, that as experience increases, the minimum B<sub>1</sub> content may be stepped up appreciably.

TABLE 3.—*Influence of tempering on the thiamin content of experimentally milled flours for a sample of Manitoba 1° wheat milled at two different moisture levels.\**

Moisture of wheat at rolls, %	Milling time, min.	Flour actual	Yield, % by difference	Flour ash, %	Thiamin per lb. of flour, i. u.
12.5	22	74.7	76.2	0.54	365
	22	72.4	74.4	0.62	353
	34	74.6	77.2	0.61	411
	34	75.1	77.4	0.65	437
	44	74.9	78.6	0.71	479
	45	76.9	78.6	0.71	506
15.5	22	73.4	74.8	—	254
	22	71.6	73.9	0.51	229
	34	69.8	73.6	0.53	292
	33	73.3	75.7	0.53	300
	45	72.1	75.5	0.59	298
	42	71.4	75.3	0.59	299

\*Original moisture of wheat 10.8%. Wheat milled on basis of 1,730 grams of dry matter per sample. Thiamin content of wheat 655 i. u. per pound.

While it is admitted that the production of an acceptable white flour carrying the above-mentioned minimum of thiamin per pound offers a few practical difficulties, we are firmly of the opinion that if the miller will apply his ability and skill to the problem, these difficulties will be readily overcome. In most mills there appear to be practical difficulties in the way of milling at a lower moisture content than that commonly followed so it may be necessary to modify or abandoned this idea of so-called "dry milling" and seek a solution somewhere further along in the milling process. Many Canadian millers are now grappling with the problem and appear to be making considerable progress. The results obtained from dry milling have suggested, among other things, that the principle involved may be applied to certain mill stocks which may be made to give up much of their thiamin content to the finished flour. Investigations along these lines are proceeding, and it is confidently hoped that there will be on the market in Canada in the near future an officially approved high vitamin B white flour and high vitamin B white loaf carrying the official brand or seal of the Government and duly protected by machinery already available. The interest taken in Canada in the whole problem of better nutrition suggests that the time is most opportune to take such steps as will restore our bread to the position which it was always intended to hold, *viz.*, that of being the real "Staff of Life".



## THE SOIL SCIENTIST LOOKS AHEAD<sup>1</sup>

FIRMAN E. BEAR<sup>2</sup>

POSSIBLY as good an approach as any to this topic would be to define the words, "soil" and "scientist," in order that we may know who it is that is looking ahead and at what he is looking.

Some years ago I learned the original meaning of the word "soil" by a peculiarly circuitous route. Having finished some work which I had been doing in Berlin, I decided to return home by way of Russia. In order to obtain permission to enter that country it was necessary to supply the proper credentials. These credentials recorded, among other facts, that I was a Professor of Soils. This title was duly translated into Russian and, when the permit was finally granted me, had been retranslated into English. From this permit I learned that I was a "Professor of the Bottom." This last word is a literal translation of the Latin *solum*, from which the English word, "soil" is believed, by some authorities, to have been derived.

Before going to the bottom of this subject, I would like to remind you that the word "science" had its origin in the Latin *scire*, meaning "to know." But a clearer idea can be obtained of what science should mean to those of us who deal with the soil, and of what the soil scientist's obligation to society consists, by reading what my dictionary says on this subject. I quote:

"Science does not, like the mechanic arts, make production its chief aim, yet its possible productive application in the arts is a constant stimulus to scientific investigation; the science, as in the case of chemistry or electricity, is urged on to higher development by the demands of the art, while the art is perfected by the advance of the science."

Soil science has for its specific task the development of more exact knowledge about the soil to the end that more effective use shall be made of it. Now there are a great variety of uses to which soil may be put. I would welcome into our fellowship the scientist who thinks of soil as an economic asset; the scientist whose primary concern with the soil is as a foundation material for highways; the scientist who is interested in soil separates as employed in the ceramic arts; the scientist who views soil as a natural resource to be conserved; the scientist whose problem is that of fitting the soil to the plant, or the plant to the soil; and the scientist who dwells on the land and uses it as a direct means of making a living.

But, for the purpose of this discussion, I propose to confine my attention to those whose primary concern with the soil is, in some manner, related to its agricultural use, and to limit myself still further by including only those who make their living off the soil, rather than on it. I am thinking of those technically trained men

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who, by seeking to hold the soil in place, as with a terrace; by digging down deeply into it, as with an auger; by tearing it to pieces, as with an acid; by examining its mineral components, as by the use of X-rays; by studying the life in the soil, as with a microscope; by testing its nutrient-supplying powers, as with a corn plant; by measuring its physical properties, as with a dynamometer; by adjusting its chemical properties to specific crop needs, as by the use of lime and fertilizers; by studying the economic and social aspects of its use, as when a given acreage of it is operated as a farm unit; or by employing any other scientific means, are trying to find the answers to the problems that continue to present themselves to the tillers of the soil.

Apply all of these procedures to all of the soils of the United States and one has a problem the immensity of which is difficult to visualize. For there are 1,903,000,000 acres of land—approximately 1 acre for every man, woman, and child on earth—within the boundaries of the 48 states and the District of Columbia. And to this body of soil there is depth, as well as length and breadth, and a fourth dimension—Time—to be measured. Soil is a dynamic, living, moving entity, and the soil scientist must consider it from that point of view.

One of the best methods of getting a concept of the size of the problem which has been assigned to the soil scientists of this country is by travel. One should go to see the soil as it exists in place; as its depths are laid bare by the plow, assisted by wind and water; as its properties are manifested in the plants that grow upon it; as its qualities are reflected by the physical characteristics of the animals that graze over it; and as it puts its stamp on the minds of the men who manage it. But if one spent his whole life in travel and stopped on the brink of the Grand Canyon to get a glimpse of the part played by Time, he would not have attained more than a mere fraction of his desire.

Fortunately, there has long been the spoken word by which knowledge of the soil, and of the potentialities of its use and abuse, have been passed on from one generation of farmers to the next. I am constantly being re-impressed with the inherent wisdom of those who live on the land—who deal with the plants that send their roots down into the soil, and with the livestock to which these plants are fed. I am convinced that a systematic compilation and analysis of the observations of the intelligent farmers of this country would yield as large a return on the investment in soil science as could be obtained by the same sum in any other manner.

We need to be reminded that a much larger number of scientifically trained men are operating farms and making their living entirely from the land than are employed in scientific research in soils under state and federal auspices. Many of these men are graduates of the best technical schools in America. Some of them have been trained in the finest soil science laboratories of our several agricultural colleges. These down-to-earth scientists are putting into practice the best that our profession has to offer in the way of working knowledge. An ever-increasing number of the larger farm enterprises are equipping soil- and plant-testing laboratories and employing technically

trained men to man them. The findings of these laboratories are being constantly checked up in the field. It is no longer necessary for the managers of these farms to knock at our laboratory doors. They can go ahead without us.

I emphasize this point because I think there is great need for soil scientists to recognize that they no longer have a monopoly on the knowledge of the soil. They might also profit by a realization that there is abundant opportunity for them to learn as well as teach. And, in learning, they would do well to sit at the feet of the scientifically minded farmer who, of necessity, applies the economic yardstick to our laboratory science before he puts in into practice in the field. Somehow, we should take greater advantage of what these modern Jethro Tulls know, and provide some means by which their observations could be permanently recorded. Their findings should be made a part of the living literature of the science of the soil.

It is in this literature that one finds the soil scientists' answers to the question as to what is the job to which they have been assigned. I happen to be an editor of one of the world's most important technical journals dealing almost entirely with soil investigations. Since the first of January this year, 69 papers, covering approximately 800 pages, have been published in that journal. Of these, nine have dealt with new laboratory apparatus; nine more with some phase of the phosphorus problem; seven with ion relationships in soils and plants; six with organic-matter effects; five with soil structure; four with specific soil microorganisms; three each with colloidal constituents, trace elements, and pH problems; two each with base-exchange phenomena, moisture relationships, potassium release, fertilizer practice, and the use of radioactive elements; and one each with such diverse topics as synthetic soil, nitrogen economy, tissue-testing for nutrient deficiencies, examination of secondary minerals, erosion control, oxidation-reduction phenomena, the fate of biologically fixed nitrogen, the space factor in crop production, and studies of sodium and selenium in soils and plants.

But these papers represent the results of work which was begun some years back. I have no way of knowing what new types of work are being undertaken, what new methods are now being employed, or what new discoveries are about to be announced. The facts which continue to impress me, as editor, are these: The ever-widening scope of interest on the part of soil scientists; the ever-quickenning rate of application of the findings of pure science; the ever-growing excellence of the papers that are presented for consideration; and the grace with which critical reviews are given, received, and acted upon. Competition is cordial, but keen. There is every reason to believe that the rate of progress in soil science during the next quarter of a century will be very rapid.

Yet, I want to point out that there is such a thing as luxury—even in scientific work in soils. It may be entirely proper and desirable that a certain number of highly capable soil scientists be permitted to investigate any subject which arouses their curiosity, no matter how far it may be removed from practice. But—and especially in in times of national emergency like these—the question will be raised

as to just how much of this type of research the nation can afford. By and large, it is our job to find the answers to immediate, pressing problems with which farmers are confronted. And, for such investigations as have for their purpose a look into the far distant future, there should be justification in terms of some practical use to which it is hoped that the findings may ultimately be put by the man on the land.

May I remind the soil scientist that agricultural colleges came into existence because those in charge of higher learning looked to the heavens for inspiration, rather than to the earth. But the agricultural chemists soon became intrigued with the more popular discoveries in human nutrition, and separate departments of soils had to be organized. In due time the soil chemists became so immersed in their test-tube technic that they forgot about the farmer, and the Soil Conservation Service came into being. And that Service is now so enmeshed in its own machinery that some new agency may have to be organized to take its place. The point is, in proportion as the work that we now do can find no justification in practice, new agencies will spring up to take over our job.

For those who are not disturbed by the possibility of being replaced by a more efficient type of soil scientist, I would suggest a few days in the field in company with a capable county agricultural agent. It has been my experience that one's buoyant feeling of self-sufficiency often gives way to a sinking sensation of incompetency when he is brought face to face with intelligent farmers whose feet are firmly planted on their own soil.

In order to clear up any possible misunderstanding of what I have in mind, I shall place before you some of the practical problems which now present themselves for solution:

1. Can the vegetable grower substitute chemicals for manure and sod crops, now that the motor has replaced the horse and there is no longer any manure to apply to the land, nor any need for hay?
2. Should the farmer of podsollic soils place his primary dependence upon a well-limed and well-fertilized A horizon, or should he take measures to tap the nutrient and water supplies that are stored in the B horizon?
3. Would it be worth while to attempt to develop deep-rooted grasses with highly fibrous root systems that would serve as desiccating agents for improving the structure of heavy soils of humid areas?
4. When the plant physiologist has laid down the specifications for a nutrient supply that will produce a perfect tomato plant, can the soil scientist guarantee the delivery of these nutrients, in the correct ratios, in the field?
5. Is it not possible that a lack of proper nutrient balance in the soil is the primary reason for the growing tendency of crop plants to be subject to the attacks of the microorganisms of disease?
6. Now that portable irrigation systems are available for use on a large scale in humid areas, can the soil physicist devise an instrument which will automatically notify the farmer when extra water should be applied?

7. To what additional economic uses can air-nitrogen fertilizers be put when the price of this nitrogen falls to 5 cents a pound, after the Second World War is over?
8. Have soil chemists anything to suggest as an absorbent for the urine and feces in dairy barns in areas where the cow population is high, and where the supply of straw for this purpose is no longer adequate?
9. Will it pay a vegetable farmer to rest his land by taking it out of cultivation for 1 year or longer and, if so, just what would be the resting procedure?
10. Isn't there a better system than clean cultivation that can be put into effect in peach and cherry orchards?
11. Are soil chemists well enough informed to be able to advise a farmer whether he does or does not need boron for the most successful production of a specific crop on a given soil?
12. Would it be practicable to utilize the enormous peat deposits of this country as sources of the needed organic matter on nearby soils?
13. To what extent can deep placement of liming materials and fertilizers be utilized as an aid in overcoming drouth effects?
14. Have soil and crop scientists given adequate consideration to the development of cropping systems by which plowing the land and cultivating the soil can be dispensed with?
15. Now that the power needs of the farm are no longer dependent upon the pulling-capacity of a team of horses, are there any new possibilities in the use of pan-breaking, subsoiling, mole-draining, and similar types of machinery on heavy soils?
16. Have soil technicians fully explored the possibilities of using sewage as a source of water and nutrients for the large-scale production of irrigated crops on sand areas which can be reached by pipe-lines from our larger cities?
17. Haven't our colloidal chemists the ingenuity to devise a quick-test procedure for determining the present state of tilth of a soil in relation to that to which it could be economically improved in practice?
18. Would it be worth while for market gardeners to attempt the collecting, on a large scale, of leaves, tree trimmings, vine clippings, wild growth on waste areas, and similar organic materials for use on their land?
19. Isn't it about time to consider fertilizers in terms of all of the cations and anions they contain, rather than merely in relation to their content of nitrogen, phosphoric acid and potash?
20. Are soil conservationists sure that the great American Desert is being held within its present bounds, or will it continue to spread over large areas of surrounding land?

These are merely examples of many practical problems with which the soil scientists of this country are faced and for which they must find answers, or they will be pushed aside by some group of men who



can. This does not mean that laboratory science must be abandoned, but rather that it must be supplemented by field science. There is a great dearth of well-trained men who combine the qualities of the scientist and the practitioner. They need to have the viewpoint both of chemistry and engineering. In fact, I believe that the time has arrived for the development of curricula in soil engineering at our agricultural colleges.

If I could be a young man again, I would like nothing better than to start over in this field of endeavor. I would want to start back a little farther in the pure sciences and to take a little longer to prepare myself in the fundamentals of mathematics, physics, chemistry, and biology. But I would want, also, to spend more time in the field actually working with the soil; running it through my fingers; watching it roll off the moldboard; studying the location of the roots of plants in it; and talking and learning from the farmers who live on the land, and earn their bread from it by the sweat of their brows.

# MANGANESE STATUS OF SOME IMPORTANT OHIO SOIL TYPES AND UPTAKE OF MANGANESE BY KENTUCKY BLUEGRASS<sup>1</sup>

MALCOLM H. McVICKAR<sup>2</sup>

RECENT years have seen an increase in the number of elements listed as essential to plant growth. Manganese has been added to the list and many cases of manganese deficiency symptoms have been reported. Although deficiency symptoms have been observed under Ohio conditions in only a few cases, it has not yet been demonstrated that an adequate supply of this element for maximum plant growth exists in the more important soil types of the state, or whether the amount of this element contained in crops grown on these soils is sufficient to supply the needs of farm animals or of man.

It was felt that a study of the manganese status of the A horizon of several of the more important soils of the state and the manganese uptake by bluegrass grown on these soils would give an insight into the question. It was thought that the results might serve to indicate probable situations warranting further investigations with this or other trace elements.

Because an exhaustive review of the literature concerned with the importance of manganese in agriculture would be voluminous and superfluous, only those references dealing specifically with the amount of manganese in soils and plants (especially grasses) will be cited.

The manganese content of the numerous soil types listed in the Atlas of American Agriculture (5)<sup>3</sup> show a wide variation. Carlyle (1) found that Texas clays were higher in manganese content than were sands and sandy loams. McHargue, Roy, and Pelphey (6) reported that bluegrass carried eight times more manganese than red clover. Piper (7), Jacobson and Swanback (3), Leeper (4), and Gilbert (2) found the manganese uptake by plants to be greater on the more acid soils.

## MATERIAL, PROCEDURE, AND METHOD

The soils employed for the study, with the exception of Muskingum silt loam and Trumbull silty clay loam, had been taken from their natural habitat in 1926 in 8-inch layers and transferred to the University farm at Columbus, Ohio, where they were placed in sewer tiles (24 inches deep and 30 inches in diameter) sunk into the ground, the layers of soil being kept in their natural order. All soils were cropped to a rotation of corn, oats, wheat, and hay without lime or fertilizer treatment for several years and then lay idle. In June of 1936, representative

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 128.

samples of Muskingum silt loam and Trumbull silty clay loam were added to the group.

All soils were sampled to a 4-inch depth with a cork borer on September 4, 1936. Twenty borings were taken for each soil type. The samples were dried, pulverized, and later analyzed for total manganese by a colorimetric method (8) and for replaceable manganese and manganese as  $MnO_2$  according to the method of Piper (7). The so-called "available" manganese is the sum of replaceable manganese and manganese as  $MnO_2$ . Directly following the sampling of the soils, the plots were seeded to Kentucky bluegrass which was harvested in June, 1937, dried, ground, and analyzed by the colorimetric method (8).

Results of chemical tests on these soil samples must not be considered strictly comparable with those for soil samples under natural conditions since the sewer tiles were provided with no means of surface drainage. All rain falling upon them was forced to percolate through the profile. This lack of drainage would favor water saturation and reduction processes which probably resulted in a higher replaceable manganese content than would exist under natural conditions. On the other hand, this forced percolation may have resulted in more than normal leaching.

The chemical results reported herein are the average of duplicates.

### RESULTS

In Table 1, results from the detailed chemical study of the soils employed in the research and of the vegetation produced upon those soils are reported in the order of the content of total soil manganese, the highest concentration heading the column.

TABLE 1.—*Manganese content of soil types and of bluegrass grown on these soil types.\**

Soil type	Soil manganese, p.p.m.				Manganese content of bluegrass, p.p.m.
	Total	Replaceable	As $MnO_2$	Available	
Muskingum silt loam....	2,270	127	157	284	—†
Cincinnati silt loam....	1,820	161	147	308	1.15
Crosby silt loam.....	1,770	124	211	335	1.34
Clyde silty clay loam....	1,760	226	114	337	2.64
Trumbull silty clay loam..	1,720	588	237	825	11.44
Wooster silt loam.....	1,680	151	197	348	3.60
Upshur clay.....	1,660	119	67	186	3.78
Bellefontaine silt loam...	1,630	154	289	443	3.91
Brooke clay.....	1,340	79	177	256	—†
Tyler silt loam.....	1,110	153	130	283	5.99
Clermont silt loam.....	1,030	236	15	251	13.57
Miami silty clay loam....	970	137	18	145	0.62
Blanchester silt loam....	650	65	290	355	1.36
Highest.....	2,270	588	290	825	13.57
Lowest.....	650	65	15	145	0.62
Average.....	1,493	178	157	335	4.49

\*Parts per million of oven-dry soil and moisture-free bluegrass.

†Seedings failed.

"Available" manganese is included because of Piper's (7) finding that growing plants depend upon both replaceable manganese and manganese as  $MnO_2$  as a source for this element.

Soils derived from similar parent materials but subjected to two degrees of leaching during soil formation were compared as regards their manganese contents. Four soils for this grouping are listed in Table 2. The first pair was derived from Late Wisconsin Limestone Drift and the second pair from Early Wisconsin Limestone Drift. The first soil in each pair is the less leached. The more highly leached soils were lowest in all reported forms of manganese, except in the case of Clermont silt loam which was much higher in total and exchangeable manganese than the comparable less leached soil, Blanchester silt loam.

TABLE 2.—*Manganese content of comparable pairs of soils selected to represent two degrees of leaching but derived from similar parent materials.\**

Soil type	Soil manganese, p.p.m.			
	Total	Replaceable	As $MnO_2$	Available
Late Wisconsin Limestone Drift				
Clyde silty clay loam.....	1,760	226	111	337
Miami silty clay loam.....	970	137	18	145
Early Wisconsin Limestone Drift				
Blanchester silt loam.....	650	65	290	355
Clermont silt loam.....	1,030	236	15	251

\*Less leached soil first in each pair.

The surface layer of Clermont silt loam is underlain with a tight, sticky, impervious sub-stratum. In early spring, this soil is very wet, but by mid-summer it is extremely dry. The alternate wetting and drying is associated with alternating oxidation and reduction processes. As a result, numerous iron and manganese concretions are formed. The presence of these manganese concretions probably explains the high total manganese content in the A horizon of this soil.

The soils included in this study also afforded an opportunity to study the effect of parent material upon the manganese contents of the soils. Data for this study are presented in Table 3. There appears to be no definite correlation between the manganese contents of these soils and the character of their parent material.

Although no definite relation between manganese content of bluegrass and any of the reported forms of manganese in the soils as such is apparent, Miami silty clay loam carried the lowest amount of manganese in grass, second lowest in total, and fourth lowest in available manganese. Likewise, Trumbull silty clay loam was second highest in amount for grass, highest for total and replaceable, third highest in manganese dioxide, and highest in available manganese. On the contrary, Clermont silt loam carried the highest in grass, third lowest in total, second highest in replaceable, lowest in manganese dioxide, and about average in available manganese.

TABLE 3.—*Manganese content of comparable soils formed under similar drainage conditions but from different parent materials.*

Soil type	Soil manganese, p.p.m.			
	Total	Replace-able	As MnO <sub>2</sub>	Avail-able <sup>*</sup>
Good Drainage				
Bellefontaine silt loam (Late Wisconsin Limestone)....	1,630	154	289	443
Wooster silt loam (Late Wisconsin Sandstone).....	1,680	151	197	348
Muskingum silt loam (Residual Sandstone).....	2,270	127	157	284
Fair Drainage				
Brooke clay (Residual Limestone).....	1,340	79	177	256
Upshur clay (Residual Shale)	1,660	119	67	186
Poor Drainage				
Clyde silty clay loam (Late Wisconsin Limestone)....	1,760	226	111	337
Trumbull silty clay loam (Late Wisconsin Sandstone and Shale).....	1,720	588	237	825

\*Sum of replaceable manganese and manganese as MnO<sub>2</sub>.

The manganese content of bluegrass grown on soils below pH 5.0 was as an average on a moisture-free basis 7.68 parts per million, while that of vegetation from soils of pH 5.0 or above was only 1.84 parts per million (Table 4). There is evidence (7) that manganese

TABLE 4.—*Manganese content of Kentucky bluegrass as related to soil reaction.*

Soil type	pH	Manganese in bluegrass, p.p.m.
Soils with pH Below 5.0		
Clermont silt loam.....	4.4	13.57
Trumbull silty clay loam.....	4.6	11.44
Tyler silt loam.....	4.6	5.99
Upshur clay.....	4.8	3.78
Wooster silt loam.....	4.9	3.60
Average.....	—	7.68
Soils with pH 5.0 or Above		
Blanchester silt loam.....	5.0	1.36
Cincinnati silt loam.....	5.0	1.15
Bellefontaine silt loam.....	5.3	3.91
Miami silty clay loam.....	5.4	0.62
Clyde silty clay loam.....	5.7	2.64
Crosby silt loam.....	5.9	1.84
Average.....	—	1.84

becomes unavailable by conversion to the higher oxides associated with the higher pH range. The data presented in Table 4 are in accord with such findings.

In Table 5, the soils are classified into poorly drained gray soils, low in organic matter; fair to well-drained light-colored soils; and poorly drained dark soils, high in organic matter. Results in Table 5 are in agreement with the findings of Piper (7). The average manganese uptake by bluegrass in parts per million was 10.33 on the poorly drained gray soils, low in organic matter; 2.12 on fair to well-drained, light-colored soils; and 2.00 on poorly drained, dark-colored soils.

Under conditions of poor aeration, reducing processes are more prevalent than those of oxidation, causing a larger proportion of the soil's manganese to be in divalent form. The solubility of the divalent form is greater than that of the more highly oxidized forms and therefore should be more available to plants.

TABLE 5.—*Manganese content of Kentucky bluegrass as related to soil aeration.*

Soil type	Manganese in bluegrass, p.p.m.
Poorly Drained Gray Soils, Low in Organic Matter	
Clermont silt loam.....	13.57
Trumbull silty clay loam.....	11.44
Tyler silt loam.....	5.99
Average.....	10.33
Fair to Well-drained, Light-colored Soils	
Miami silty clay loam.....	0.62
Bellefontaine silt loam.....	3.91
Cincinnati silt loam.....	1.15
Wooster silt loam.....	3.60
Average.....	2.12
Poorly Drained Dark-colored Soils, High in Organic Matter	
Clyde silty clay loam.....	2.64
Blanchester silt loam.....	1.36
Average.....	2.00

It is, however, impossible to say with certainty that the increase in manganese uptake is due to soil reaction or to poor aeration associated with poor drainage and low organic matter content, since the three soils producing grass with highest manganese content are included in both the low pH and poorly drained, low organic matter content group. It is probable that both factors may be important.

#### SUMMARY AND CONCLUSIONS

This research comprised (a) manganese determinations, total, replaceable, as  $MnO_2$ , and available, of the A horizon of several im-

portant Ohio soil types; (b) manganese uptake by Kentucky bluegrass grown on these soils; and (c) observations of the relationships between manganese content of soils and their genetic characteristics and manganese content of Kentucky bluegrass and soil reaction and soil aeration.

A wide variation in all forms of determined manganese in the soil was observed.

In a general way, the more highly leached soils were lower in all forms of manganese. An exception appeared in the instance of Clermont silt loam, a highly leached soil which showed a total and replaceable manganese content much higher than its comparable soil, Blanchester silt loam. This is probably due to an accumulation of manganese in the form of concretions, a well-recognized feature of the Clermont silt loam profile.

The nature of a soil's parent material could not be used as an index of the manganese content of that soil.

Grass grown on Miami silty clay loam carried the lowest manganese content. The soil on which the grass was grown showed the second lowest in total manganese, fourth lowest in replaceable manganese, second lowest in manganese dioxide, and lowest in the available form. Likewise, Trumbull silty clay loam was second highest in manganese content of grass, highest in total and replaceable manganese, third highest in manganese dioxide, and highest in available manganese. On the contrary, Clermont silt loam carried the highest manganese content in grass, third lowest in total, second highest in replaceable, lowest in manganese dioxide, and near average in available manganese.

The manganese uptake in parts per million by bluegrass on soils below pH 5.0 averaged 7.68 as compared with 1.84 on soils of pH 5.0 or above.

Aeration, as associated with drainage, influenced the manganese uptake of Kentucky bluegrass. Bluegrass growing on poorly drained soils low in organic matter contained, as an average, 10.33 parts per million of manganese, while that from poorly drained dark-colored soils, high in organic matter, contained 2.00 parts per million.

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## COMPARATIVE YIELDS OF TRANSPLANTED AND DIRECT SOWN RICE<sup>1</sup>

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TWO general methods of sowing or planting rice are used throughout the world. These are direct seeding and transplanting. In the United States the commercial rice crop is all direct sown and is grown in essentially the same manner as wheat, oats, and barley, except that the land is submerged during most of the growing season. Farm machinery is used in preparation of the land, in seeding, and in harvesting the crop. In the Orient machine methods are generally not employed for the farms usually are too small. Work animals, when available, are used in preparing the land. The rice usually is sown broadcast in small, well-prepared beds where it is given the best of care during the 25 to 50 days that it is grown there. The seedlings are then pulled and transplanted by hand in the field. Before transplanting, the fields are submerged a few inches and the soil is stirred into a loose mud. One to five seedlings are placed in hills spaced 3 to 6 inches apart in rows 8 to 12 inches apart.

The high yields of transplanted rice often obtained under intensive culture in certain countries have led to a more or less common belief that yields of transplanted rice generally are higher than those from direct seeding. It seemed desirable to compare the yields of rice planted by the two methods in the United States.

An experiment was begun in 1937 in which three varieties at three rice experiment stations in the southern states and two varieties at the California rice experiment station were direct sown and transplanted during a 3-year period. The results are reported herein.

### LITERATURE

All Oriental rice that enters commercial trade, according to Copeland (7, page 226),<sup>3</sup> is grown by the transplanting method. The literature dealing with the preparation and care of seedbeds, effect of age of seedlings when transplanted, number of seedlings per hill, and the space between hills and rows on yield is extensive. Studies also have been reported in which the yields of rice from direct seeding and transplanting are compared.

In Egypt higher yields were reported from direct sown than from transplanted rice (1, 8). At the present time, however, part of the rice acreage in

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 136.



Egypt is transplanted which indicates that even there, under certain conditions, transplanting is considered to be feasible.

In Italy a portion of the rice crop is transplanted and Tempary (26) reported that higher yields are obtained from this method than from direct seeding. In Spain, also, transplanting is a common practice, and the acre yields are high.

In Ceylon, Lord (18) reported that transplanted rice produced 31% more than that sown broadcast. Joachim, *et al.* (11) found that rice produced on transplanted fields contained a higher percentage of phosphoric acid and slightly more nitrogen than that from broadcast fields. Haigh (10) states that, "The most important determiner of acre yield is spacing, which overshadows individual plant performance, so that a bigger crop is produced from a large number of small plants than from a small number of large ones." In hills spaced 4×4 inches apart there was no advantage in using more than one seedling per hill, but at wider spacings three seedlings per hill had an advantage.

In Java, van der Goot (9) found that some late and early varieties produced the best yields from transplanting seedlings 40 days old, whereas other early varieties produced about the same yield from seedlings of various ages.

In the Philippines, Jacobson (12) suggested that the proper spacing and number of seedlings per hill should be determined for each variety. Early varieties may require less space than late varieties. He also reported (13) that a medium-early variety gave higher yields from seedlings 20 days old than from seedlings 30 or 40 days old at transplanting time. However, in another test, the highest yield of early rice was obtained from transplanting seedlings 40 days old, and that of midseason and late varieties from 60-day-old seedlings. Rodrigo (21) reported that space had a marked effect on the number of tillers produced per plant, ranging from 1.49 to 11.65 for single plants spaced 10×10 and 40×40 centimeters apart. Camus (5) stated that on poor soils more space between hills is needed than on richer soils, and the space required for best yields also depends upon the date of maturity of the rice. With respect to age of seedlings for transplanting, he recommended for varieties maturing in less than 120 days seedlings about 25 days old; for varieties maturing in 120 to 145 days, seedlings 30 days old; for varieties maturing in 145 to 170 days, seedlings 30 to 35 days old; for varieties maturing in 170 to 180 days, seedlings 35 days old; and for still later varieties, seedlings 40 days old. Calvo (4) found that transplanted rice produced more tillers than rice sown direct, and that as the number of seedlings per hill increased from 1 to 4 the number of tillers per plant decreased from 9.17 to 3.20. Bautista (3), without supporting data, stated that transplanted rice yields from 15 to 20% more than that sown broadcast.

In India, Ramiah and Row (20) reported that the optimum spacing in hills varies with the soil and ranges from 4½ to 9 inches for varieties that require more than 150 days to mature and 4 inches for early varieties. Ramiah (19) also obtained higher yields from transplanted than from broadcast rice. However, sprouted seeds dibbled in rows on puddled soil yielded as well as or better than transplanted rice. The number of tillers produced per plant, according to Thompsonstone (27), depends on the available space and the maturity of the rice. Alam (2) found that the age of the seedlings transplanted had no appreciable effect on yield, and that four seedlings per hill with the hills spaced 9 inches apart gave the highest yields. Sengupta (22) obtained higher yields from doubled transplanting than from single transplanting, and Codd (6) reported similar results in British Guiana.

Sherrard (25) in India reported that transplanted rice yielded more than that direct sown and that essentially the same yield was obtained from seedlings grown on moist and puddled seedbeds. There was a progressive increase in yield from seedlings transplanted when 20, 30, and 40 days old, respectively. Best results were obtained from transplanting two to six seedlings per hill with the hills spaced 6 to 9 inches apart. Joshi (14) observed that seedlings ranging in age from 16 to 40 days gave maximum yields. Reducing the number of seedlings per hill did not increase the number of tillers unless the hills were spaced farther apart. He also found that five or six *dry* seeds sown in hills regularly spaced gave as high or somewhat higher yields than transplanted rice. This led him to suggest that the principle underlying transplanting seems to be more for adjustment with environmental and economic requirements than for any physiological advantages. Sethi (23) enumerated the following reasons for transplanting: (a) Increased yields, (b) requires less seed, and (c) gives rice of better quality.

In China, Shen (24) compared the direct seeding and transplanting methods in nursery tests and when the rice was sown on the same date found little difference in yields. However, much more labor was required by the transplanting method.

In Japan transplanting is the common method, but high yields also are obtained from direct seeding. Yamaguchi (28) claimed that better seedlings are produced on a saturated seedbed than on seedbeds submerged either shallow or deep. According to Katayama (15, 16), the space and length of time that seedlings remain in the seedbed affects the number and point of origin of tillers; also, delayed transplanting resulted in later heading, a reduced weight of the panicles and kernels, and an increased number of premature heads. Kojima and Chosokabe (17) observed similar effects in Manchuria; also, that late varieties were less seriously affected by prolonged growth in the seedbed than were early varieties.

These reports on the comparative yields of rice direct sown and transplanted, on the care and preparation of seedbeds, the best age of seedlings for transplanting, the proper number of seedlings per hill, the space between hills, and the effect of these factors on yields are rather conflicting. This is due, no doubt, to the fact that the results were obtained under different environmental conditions, on soils of various types and fertility, and with varieties that differ in their physiological responses.

#### MATERIAL AND METHODS

The rice varieties grown in the present experiment were Caloro (a short-grain variety), Early Prolific (a medium-grain variety), and Arkansas Fortuna (a long-grain variety). In the southern states, Early Prolific matures in 127 days, Caloro in about 136 days, and Arkansas Fortuna in 142 days from seeding. In California, Caloro and Early Prolific require from 12 to 15 days longer to reach maturity than in the South.

The varieties were grown in three-row plots 1 rod long with the rows spaced 1 foot apart, and the center row of each plot was harvested for yield. Each variety and treatment was grown in four replications. The treatments within varieties were paired so that plots direct sown were adjacent to transplanted plots. The order within pairs and of the varieties was distributed at random.

The rice direct sown was seeded at the usual time with a hand planter at the rate of approximately 90 pounds per acre at the southern stations and 130 pounds in California. At the same time a small adjacent area was sown to supply seed-

lings for transplanting. At the time of first irrigation the seedlings, ranging from 4 to 5 weeks in age, were pulled and three or four seedlings were transplanted in hills spaced about 6 inches apart in the rows spaced 12 inches apart. The plants in rows direct sown were not thinned, as the purpose of the experiment was to compare the yields of transplanted rice with that grown by American methods.

### EXPERIMENTAL RESULTS

The yields obtained at all four stations are given in Table 1.

TABLE 1.—Average annual and 3-year average yields of three varieties of rice direct sown and transplanted at four stations.

Year	Acre yield, bushels							
	Caloro		Early Prolific		Ark. Fortuna		Average	
	Direct	Trans-planted	Direct	Trans-planted	Direct	Trans-planted	Direct	Trans-planted
Stuttgart, Ark.								
1937	51.2	37.2	46.8	40.1	45.2	32.7	47.7	36.7
1938	49.7	53.8	38.0	45.4	51.1	42.6	46.3	47.3
1939	45.4	44.6	26.5	29.7	49.7	37.3	40.5	37.2
Av. ....	48.8	45.2	37.1	38.4	48.7	37.5	44.8	40.4
Crowley, La.								
1937	48.0	39.2	55.6	42.3	39.1	31.8	47.6	37.8
1938	21.0	23.2	21.8	17.8	27.3	18.3	23.4	19.8
1939	42.1	21.4	42.1	24.4	38.3	25.7	40.8	23.8
Av. ....	37.0	27.9	39.8	28.2	34.9	25.3	37.3	27.1
Beaumont, Tex.								
1937	41.2	36.8	40.8	30.6	41.3	36.5	41.1	34.6
1938	41.0	42.0	30.6	33.4	43.3	47.9	38.3	41.1
1939	31.1	30.4	25.1	21.4	33.7	33.5	30.0	28.4
Av. ....	37.8	36.4	32.2	28.5	39.4	39.3	36.5	34.7
Av. 3 south- ern sta- tions...	41.2	36.5	36.4	31.7	41.0	34.0	39.5	34.1
Biggs, Calif.								
1937	74.8	56.4	48.9	34.5	—	—	61.8	45.4
1938	120.6	82.3	82.2	55.8	—	—	101.4	69.0
1939	112.4	93.1	101.0	80.3	—	—	106.7	86.7
Av. ....	102.6	77.3	77.4	56.9	—	—	90.0	67.1

In Arkansas, Arkansas Fortuna direct sown gave significantly higher yields each year than when transplanted. The 3-year average

yield of direct sown Caloro was higher than when transplanted, but for Early Prolific the yield was slightly higher from transplanting. However, the increases were significant only in 1937 for Caloro and in 1938 for Early Prolific. During the 3-year period the yields from direct seeding exceeded those from transplanting in six of the nine comparisons, in four of which the differences were significant, whereas the yield from transplanting was significantly higher in only one test.

In Louisiana, in 1938, the yield of transplanted Caloro was higher than when direct sown, but the increase was not significant. Each year in the other eight comparisons the yields of each variety were higher from direct seeding than from that transplanted, and in six of the eight tests the increases were significant. The average yields for all varieties in the 3-year period from direct seeding were significantly higher than when transplanted.

In Texas the yield of each variety was higher from direct seeding than from transplanting in 1937 and 1939 and lower in 1938. All increased yields from direct seeding were significant in 1937 but not in 1939. The average yields of Caloro and Arkansas Fortuna in the 3-year period were not significantly different for the two methods; however, the increase of Early Prolific from direct seeding was significant. In six of the nine comparisons the yields were higher from direct seeding than from transplanting.

In California, on more fertile soil, the yields of both varieties during the 3-year period were significantly higher from direct seeding.

Of the 33 comparisons grown at the four stations during the 3-year period, the yields from direct seeding were higher than from transplanting in 26 and lower in 7. In 19 of the 26 comparisons the increases were significant, but the decreased yield in only one of the seven comparisons was significant.

The 3-year average yields of each variety and for the three varieties grown at the three southern stations and of both varieties in California were significantly higher from direct seeding than from transplanting.

In Table 2 are given the values of *F* for the items studied. The variations due to varieties, except in Louisiana; southern stations; years; varieties $\times$ southern station; varieties $\times$ years, except at Arkansas and California; and southern station $\times$ years were significant. The variation due to varieties $\times$ southern station $\times$ years was not significant.

The variations due to treatments, except probably at the Texas station; treatments $\times$ southern stations; treatments $\times$ years; treatments $\times$ varieties $\times$ southern stations; and treatments $\times$ southern stations $\times$ years were significant. However, the variations due to treatments $\times$ varieties, except at the Arkansas station; treatments $\times$ varieties $\times$ years; and treatments $\times$ varieties $\times$ southern stations $\times$ years were not significant.

#### DISCUSSION

Published results indicate that under certain conditions the yield of transplanted rice usually is higher than when direct sown.

TABLE 2.—*Values of F for the various items studied in comparison of the three varieties of rice direct sown and transplanted at four stations for 3 years.*

Item	Location				
	Stutt- gart, Ark.	Crow- ley, La.	Beau- mont, Tex.	Three south- ern sta- tions	Biggs, Calif.
Varieties.....	6.04†	1.35	58.92†	7.32†	44.64†
Stations.....	—	—	—	33.16†	—
Years.....	4.39*	56.33†	84.75†	16.81†	56.79†
Varieties X southern stations.....	—	—	—	5.65†	—
Varieties X years.....	2.62	3.18*	6.38†	5.36†	2.99
Southern stations X years.....	—	—	—	22.69†	—
Varieties X southern stations X years..	—	—	—	0.86	—
Treatments (direct sown or trans- planted).....	11.91†	64.79†	4.19	66.97†	140.70†
Treatments X varieties.....	7.79†	0.39	1.51	1.32	1.57
Treatments X southern stations.....	—	—	—	13.76†	—
Treatments X years.....	7.42†	9.45†	9.84†	17.76†	6.22†
Treatments X varieties X southern sta- tions.....	—	—	—	4.91†	—
Treatments X varieties X years.....	1.07	1.84	0.58	1.42	0.98
Treatments X southern stations X years	—	—	—	4.11†	—
Treatments X varieties X southern sta- tions X years.....	—	—	—	1.22	—

\*P less than 0.05.

†P less than .01.

Some of the advantages claimed for transplanting are (a) increased yields, (b) better utilization of available farm land, (c) better control of aquatic and other weeds, (d) requires less water for irrigation, (e) requires less seed, (f) stimulates root growth and tillering, (g) reduces lodging, (h) rice matures more uniformly and is of better quality, (i) increases the ratio of grain to straw, (j) permits the plants to receive more sunshine and a freer circulation of air which may reduce the loss from diseases and insects, (k) results in less sterility, (l) facilitates the application of fertilizers during crop growth, and (m) the crop is easier to harvest by hand methods. Of these suggested advantages some are self-evident while others are rather intangible.

In the densely populated Asiatic countries, available tillable land is limited and double cropping is a common practice. In such areas transplanting makes it possible to grow two crops each crop year, whereas only one could be grown if rice were direct sown. For example, in Japan rice land is bedded in the fall after harvest and sown to wheat or barley. These cereals do not mature in time for direct seeding of rice in the spring, but they do mature, are harvested, and the land prepared in time to receive transplanted rice; hence, transplanting is an important factor in improving land utilization. Transplanted seedlings soon become established and are better able to compete with weeds than are young seedlings.

The method of transplanting in spaced hills also facilitates weeding by hand or by mechanical means. Transplanted rice occupies the main fields for a shorter time than rice direct sown, hence less irrigation water is required to produce a crop.

Less seed is required to sow an acre by the transplanting method, but the saving in seed, even under the most favorable prices of rough rice, cannot be equal to the extra expense involved in raising, pulling, transferring, and transplanting seedlings as compared with direct seeding. An expert in transplanting can pull, distribute, and transplant an acre in from 50 to 80 hours.

Rice transplanted in rows 6 to 12 inches apart, with the plants in spaced hills in the rows, is of course more convenient to fertilize during growth and is easier to harvest by hand than are drilled or broadcasted fields. Transplanted rice probably is less inclined to lodge and the product may be of better quality than when direct sown because each plant can be harvested when mature. However, high quality, stiff-strawed varieties are now being grown by machine methods in several countries. The other advantages enumerated are evidently of much less economic importance than those just discussed.

By intensive soil cultivation and fertilization transplanted rice produces high acre yields, but with less intensive methods the yields are relatively low. Rice direct sown on fertile soil also produces high yields, whereas relatively low yields are secured under less favorable conditions. The more or less general opinion, therefore, that transplanted rice is always more productive than rice direct sown is not based on well-established facts.

At Biggs, Calif., in 1936 individual Caloro seedlings about 6 weeks old were transplanted in hills 6, 8, and 12 inches apart in rows 16 feet long, spaced 1 foot apart. Control rows were direct sown at the rate of 16 grams per row. Each treatment was replicated seven times.

The average number of tillers per row for direct seeding was 377, and for transplanting for 6-inch spacing 192, for 8-inch spacing 168, and for 12-inch spacing 139. The average yields for direct seeding were 5,032 pounds and for the 6-, 8-, and 12-inch transplanted spacings 3,789, 3,801, and 3,004 pounds per acre, respectively. In the transplanted rows there was a progressive increase in the number of tillers per plant with the increase in space between plants, but the plants even at the 6-inch spacing were too far apart to produce maximum acre yields.

It seems logical, therefore, to suggest that the principal reasons for transplanting are not necessarily increased yields, but a better utilization of land and labor in densely populated countries in which the tillable land area is limited and labor is relatively cheap.

#### SUMMARY

The varieties Caloro, Early Prolific, and Arkansas Fortuna rice were grown at the Arkansas, Louisiana, and Texas rice experiment stations, and Caloro and Early Prolific were grown at the Biggs, Calif., Rice Field Station during the 3-year period of 1937 to 1939 to compare the yields from plots direct seeded and transplanted.

The average yields of Caloro for the 3-year period from direct seeding were significantly higher than from transplanting in Louisiana and California. The average yields of Early Prolific also were significantly higher from direct seeding than from transplanting in Louisiana, California, and Texas, also for Arkansas Fortuna in Arkansas and Louisiana. None of the varieties at any of the stations produced significantly higher average yields when transplanted than when direct sown.

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## CONVERGENT IMPROVEMENT WITH FOUR INBRED LINES OF CORN<sup>1</sup>

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THE modern method of corn breeding is based on the utilization of inbred lines in some form of hybrid combination. Inbred lines of corn are more or less reduced in vigor as compared to normal varieties or hybrids. Since these lines are reduced in vigor some method of crossing is necessary to restore this vigor. This expression of hybrid vigor is greatest in single crosses between inbred lines each of which contains important genes for vigor of growth that are not found in the other.

As shown by many workers (2, 3, 7, 8),<sup>3</sup> there is a significant positive correlation between the vigor of inbred lines and their performance in crosses. However, it is necessary to test the yielding ability of an inbred line in crosses as it is impossible to classify accurately the combining ability of inbreds on the basis of their performance as inbreds. Jenkins (4) has shown that certain inbred lines were high in combining ability when tested during the early generations of selfing and that these lines maintained this character in later selfed generations. Hayes and Johnson (2) gave considerable evidence to indicate that the combining ability of inbred lines selected from  $F_1$  crosses was correlated with that of the inbred parents. Therefore, it seems that combining ability and vigor are heritable characters and that there is a positive association between these characters in inbred lines. Vigor is also a desirable character to have in inbred lines in order to maintain them more easily and economically.

The primary method of developing inbred lines has been by selection in self-pollinated lines that originated from open-pollinated varieties. Following the isolation of inbred lines and the determination of their value in crosses, it may be desirable to improve their vigor. This may be done by pedigree selection within self-pollinated progenies resulting from crosses between selected parents as reported by many workers (2, 5, 11). In addition, the backcross method may be used as a means of improving the vigor of inbred lines.

In the breeding methods involving backcrossing two methods have been used by corn breeders primarily for the purpose of improving inbred lines which have already proved desirable in hybrid combina-

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 149.

tion. The backcross method, as described by Harlan and Pope (1) in their studies with barley, is of value in the improvement of an inbred line by adding one or two simply inherited characters that it lacks. The other method involving backcrossing, known as convergent improvement, was originated by Richey (9). This method, by double backcrossing, was outlined as a means of improving each of two parental inbred lines of a single cross without modifying the yielding ability of that cross.

### REVIEW OF LITERATURE

Convergent improvement was outlined and described by Richey (9) in 1927 and experimental results were given by Richey and Sprague (10) in 1931. The method of breeding was outlined primarily in an effort to provide experimental data to test the reliability of the theory of hybrid vigor as set forth by Jones (6) in 1917. Hybrid vigor was explained by Jones on a Mendelian basis by the hypothesis that dominant linked genes for vigor, a part of which were contributed by each of the two parents, were combined in the cross. Richey (9) suggested also that convergent improvement seemed to have plant breeding value since each of the two parents could be improved in vigor by converging their genotypes without changing their combining ability. If the genes concerned are only partially dominant, it was pointed out that it should be possible to recover lines which would yield more in crosses than the original  $F_1$  cross, since the single cross between the recovered lines would be homozygous for some pairs of genes that were heterozygous in the original  $F_1$  cross.

In their experiments, Richey and Sprague (10) averaged the results for six different single crosses. In one series they studied the yield of successive generations of backcrosses when crossed with the recurrent parent, showing that in the absence of selection the yields of successive backcross progenies in these crosses should be reduced by  $\frac{1}{2}$ ,  $\frac{3}{4}$ ,  $\frac{7}{8}$ , etc., of the difference between the yield of the  $F_1$  cross and that of the recurrent parent. They found that the first backcross progeny tested in crosses to the recurrent parent yielded one-half way between the  $F_1$  cross and the yield of the recurrent parent as would be expected. However, when selection for vigor characters was practiced during the period of back pollination, they found that these backcross progenies yielded significantly higher in crosses to the recurrent parent than was expected. This increase in yield of the backcross progenies tested in crosses to the recurrent parent over the expected yield of unselected backcross progenies tested in a similar way was attributed to dominant favorable genes from the non-recurrent parent which were retained by selection during the period of back pollination.

When the various backcross progenies from one to six generations of back pollination were crossed to the non-recurrent parent, the increase in yield for each over the difference between the yield of the parents and the yield of the  $F_1$  cross was in excess of the  $\frac{1}{2}$ ,  $\frac{3}{4}$ ,  $\frac{7}{8}$ , etc., of the difference expected in each succeeding generation without selection. Although the recovered lines had not been selfed following the backcrossing program, they seemed to be improved in yield, lodging resistance, and pollen production.

They concluded also that there is a probability that some of the crosses between recovered lines will yield better than the original  $F_1$  cross since three out of six such crosses yielded more than the corresponding  $F_1$  cross probably because there is only partial dominance of some of the genes for vigor.

## MATERIALS AND METHODS

Four inbred lines were used in this study. They were developed by selection within selfed progenies from an open-pollinated variety, Rustler White Dent. C15 and C19 are crossed to produce single cross K and C16 and C20 are crossed to produce single cross I. These two single crosses are used as one of the parents in two commercial double crosses which were the first developed and released by the Minnesota Agricultural Experiment Station.

Four groups of backcrosses were studied. They were C15×C19 back pollinated to C15 in one group and to C19 in another, and C16×C20 back pollinated to C16 in one group and to C20 in another. The backcrosses were made by hand pollination except for two of the groups which were made in isolated plots in 1932.

Selection was practiced throughout the period of back pollination and selfing. Characters for which selection was made included smut resistance, lodging resistance, pollen production, ear type, resistance to ear and stalk rots, vigorous desirable plant type, and desirable seed and ear type.

In each of the single crosses six ears of the  $F_1$  were back pollinated to each parental line in 1931. From the bulk progeny of these six ears about 100 ears were back pollinated in the second backcross and 20 to 25 ears were selected and grown in ear-to-row cultures in the following year. Further generations of backcrossing were handled in a similar manner. From 8 to 10 ears were backcrossed in each selected ear culture.

From 1935 through 1938 selection in self-pollinated lines was practiced. Approximately 50 cultures of 33 plants each were grown in 1936 and 1937 and 10 to 12 ears selfed in each backcross group. In 1939 approximately 10 recovered cultures remained from each of the four original inbred lines used in the convergent improvement studies.

In 1937, after two years of selfing, about 15 of the more desirable recovered lines in each group were crossed to the recurrent and to the non-recurrent parents. These crosses were tested in yield trials at three locations. In the crosses of recovered lines to the non-recurrent parent three replications at each location were grown in randomized block trials and the original  $P_1$  cross was used as a check in each group. In the crosses of recovered lines to the recurrent parent two replications at each location were grown and the recurrent parent was used as a check in each group.

In 1939 after four years of selfing ear cultures were selected in each group of recovered lines that represented variations in combining ability when crossed with the non-recurrent parental inbred line. These recovered lines had all originated from different ears in the backcross progenies when they were first selfed in 1935. These crosses were between recovered lines of C15 with recovered lines of C19 and recovered lines of C16 with recovered lines of C20. These single crosses were tested in randomized blocks composed of three replications at each of three locations with the original  $F_1$  crosses as checks.

The method of designating the number of backcrosses in this study is similar to that used by Richey. As an example, a 2-year backcrossed culture is designated as (16×20)20. This means that the  $F_1$  single cross, 16×20, had been back pollinated to C20 for 2 years. All backcrosses are designated similarly.

## EXPERIMENTAL RESULTS

## CROSSES OF RECOVERED LINES TO THE RECURRENT PARENT

As previously described, a number of recovered lines were tested in crosses to the recurrent and to the non-recurrent parents. The yield data from these experiments will be presented in frequency distributions.

In Table 1 are given the yield data for the  $F_1$  crosses of the recovered lines with the recurrent parent in comparison with the recurrent parent. These data are given in a frequency distribution of 0, +1, -1, etc., times the standard error of the difference from the yield of the recurrent parent.

From the data in Table 1 it will be noted that each of the recovered lines of C16, C20, and C15 was improved in yield as measured in crosses with the recurrent parent as compared to the yield of the recurrent parent itself. In C19 only three of the lines were significantly higher yielding in crosses than the recurrent parent if the 5% point is used as the point of statistical significance.

The increase in yield of these crosses over that of the recurrent parent may be explained by the fact that some genes for vigor from the non-recurrent parent were retained by selection. As these lines had been selfed for 2 years they should retain most of these genes. These results do not give an indication of the number of the genes of the recurrent parent which have been retained. They should be retained without selection in each backcross generation in the series of  $\frac{1}{2}$ ,  $\frac{3}{4}$ ,  $\frac{7}{8}$ , etc. Thus, with each backcross, less of the genotype of the non-recurrent parent can be obtained if selection is not practiced. However, there does not seem to be any great difference between the 2-, 3-, and, in the one instance, the 4-year backcrosses in these studies. The results seem to indicate that important genes for vigor have been obtained from the non-recurrent parent by selection and added to the recovered lines in backcross progenies and that they were retained after self-pollination for two generations.

## CROSSES OF RECOVERED LINES TO THE NON-RECURRENT PARENT

The data on yield of the  $F_1$  crosses of recovered lines crossed to the non-recurrent parent are given in Table 2. The yield data are presented in a frequency distribution of 0, +1, -1, etc., times the standard error of the difference in comparison with the yield of the original  $F_1$  crosses.

One of these cultures when crossed to the non-recurrent parent yielded significantly higher and 11 yielded significantly lower, as measured by two times the standard error of a difference, than the original  $F_1$  cross. In each of the four groups of recovered lines some crosses yielded as well or slightly more than the original  $F_1$  cross. In many cases it seemed that sufficient of the genotype of the recurrent parent had been recovered so that these lines combined well in crosses to the non-recurrent parent. It is interesting to note that some of the progenies from the 2-year backcrosses yielded as well in crosses to the non-recurrent parent as the original  $F_1$  cross, although theoretically without selection only 75% of the recurrent parent had been retained by these lines.

TABLE 1.—*Frequency distribution of yields in classes for the recovered lines tested in single crosses to the recurrent parent.*

Frequency distribution of yield of recovered C16×standard C16 in comparison to the recurrent parent, C16												
Number of lines	Years back-crossed	Class centers of plus 6 and minus 1 times the S.E. of a difference*								Mean class	Mean yield of all crosses	Yield of recurrent parent
		-1	0	+1	+2	+3	+4	+5	+6			
5	2	—	—	—	1	1	2	—	1	+3.80±4.50	27.7±3.9	19.2±1.5
4	3	—	—	—	1	3	—	—	—	+2.75±3.21	25.2±1.7	19.2±1.5
Total 9					2	4	2		1	+3.33±3.74	26.6±2.2	19.2±1.5

\*S.E. of a difference = 2.19 bu.

Frequency distribution of yield of recovered C20×standard C20 in comparison to the recurrent parent, C20												
Number of lines	Years back-crossed	Class centers of plus 6 or minus 1 times the S.E. of a difference*								Mean class	Mean yield of all crosses	Yield of recurrent parent
		-1	0	+1	+2	+3	+4	+5	+6			
8	2	—	—	—	—	2	1	2	3	+4.75±5.24	26.7±2.8	17.5±1.3
2	3	—	—	1	1	—	—	—	—	+1.50±2.24	19.6±1.1	17.5±1.3
Total 10				1	1	2	1	2	3	+4.10±3.62	25.3±1.5	17.5±1.3

\*S.E. of a difference = 1.81 bu.

Frequency distribution of yield of recovered C15 X standard C15 in comparison to the recurrent parent, C15

Number of lines	Years back-crossed	Class centers of plus 6 or minus 1 times the S.E. of a difference*						Mean class	Mean yield of all crosses	Yield of recurrent parent
		-1	0	+1	+2	+3	+4	+5	+6	
11	2	—	—	1	6	2	2	—	—	43.3 ± 2.7
4	3	—	—	1	—	2	—	1	—	46.1 ± 6.1
Total 15				2	6	4	2	1		44.3 ± 3.4

\*S.E. of a difference = 3.37 bu.

Frequency distribution of yield of recovered C19 X standard C19 in comparison to the recurrent parent, C19

Number of lines	Years back-crossed	Class centers of plus 6 or minus 1 times the S.E. of a difference*						Mean class	Mean yield of all crosses	Yield of recurrent parent
		-1	0	+1	+2	+3	+4	+5	+6	
5	2	—	1	3	1	—	—	—	—	40.1 ± 2.3
3	3	1	—	1	—	—	1	—	—	40.4 ± 7.4
7	4	2	2	2	—	1	—	—	—	37.8 ± 3.7
Total 15		3	3	6	1	1	1			39.1 ± 2.9

\*S.E. of a difference = 2.82 bu.



Frequency distribution of yield of recovered C15×standard C19 in comparison to original F<sub>1</sub> cross

Number of lines	Years back-crossed	Class centers of plus 2 and minus 5 times the S.E. of a difference*							Mean class	Mean yield of all crosses	Yield of original F <sub>1</sub>
		-5	-4	-3	-2	-1	0	+1	+2		
12	2	—	—	—	—	7	3	1	1	57.2±2.2	58.1±1.8
4	3	—	1	—	—	2	1	—	—	53.3±5.1	58.1±1.8
Total 16			1			9	4	1	1	56.4±2.8	58.1±1.8

\*S.E. of a difference = 2.57 bu.

Frequency distribution of yield of recovered C19×standard C15 in comparison to original F<sub>1</sub> cross

Number of lines	Years back-crossed	Class centers of plus 2 and minus 5 times the S.E. of a difference*							Mean class	Mean yield of all crosses	Yield of original F <sub>1</sub>
		-5	-4	-3	-2	-1	0	+1	+2		
5	2	—	—	—	—	3	1	1	—	56.3±2.9	57.3±2.3
3	3	—	1	—	—	1	1	—	—	51.4±2.4	57.3±2.3
7	4	—	—	—	—	1	5	1	—	57.2±2.4	57.3±2.3
Total 15			1			5	7	2		55.8±2.8	57.3±2.3

\*S.E. of a difference = 3.21 bu.



The relationship of the performance of the recovered lines when tested in crosses to the recurrent parent to their performance in crosses to the non-recurrent parent was determined. The calculated correlation coefficient was found to be  $r = -0.16$ . This does not approach significance.

As previously stated, the genes for vigor obtained by selection from the non-recurrent parent are important in determining the yield of recovered lines tested in crosses to the recurrent parent. In the crosses of the recovered lines to the non-recurrent parent the genes for vigor retained automatically from the recurrent parent are probably the most important in determining the yield of the crosses. From these studies there seems to be no consistent relationship between the desirability of the genes retained automatically from the recurrent parent and the desirability of the genes retained by selection from the non-recurrent parent.

#### RECOVERED LINES SELECTED FOR INTERCROSSING

From two to four recovered lines from each of the four original lines were selected for testing in single crosses in comparison with the original  $F_1$  crosses. These lines were selected on the basis of their performance as measured by yield when tested in crosses to the non-recurrent parent. The yield of these lines tested in single crosses to the recurrent and non-recurrent parents is given in Table 3.

TABLE 3.—*Yield of the recovered lines, which were selected for intercrossing, when tested in crosses with the recurrent and non-recurrent parents.*

Culture No.	Pedigree	Yield when crossed to recurrent parent, bu. per acre	Yield when crossed to non-recurrent parent, bu. per acre	Classification for combining ability when crossed to the non-recurrent parent
C16	Rustler inbred	19.2	—	
686	(16×20) 16 <sub>2</sub>	28.3	45.5	Low
703	(16×20) 16 <sub>3</sub>	26.9	54.0	High
C16×C20	Original $F_1$	—	52.0	
C20	Rustler inbred	17.5	—	
716	(16×20) 20 <sub>2</sub>	25.2	46.1	Intermediate
719	(16×20) 20 <sub>3</sub>	22.9	37.0	Low
720	(16×20) 20 <sub>3</sub>	30.5	51.7	High
739	(16×20) 20 <sub>1</sub>	20.3	50.4	High
C16×C20	Original $F_1$	—	50.0	
C15	Rustler inbred	35.4	—	
757	(15×19) 15 <sub>2</sub>	44.2	61.2	High
772	(15×19) 15 <sub>2</sub>	43.9	55.8	Intermediate
779	(15×19) 15 <sub>2</sub>	48.4	59.7	High
C15×C19	Original $F_1$	—	58.1	
C19	Rustler inbred	36.3	—	
789	(15×19) 19 <sub>2</sub>	40.0	55.1	Intermediate
822	(15×19) 19 <sub>4</sub>	44.3	57.9	High
827	(15×19) 19 <sub>4</sub>	39.7	61.0	High
C15×C19	Original $F_1$	—	57.3	

As noted in Table 3, these recovered lines were classified into high, intermediate, and low combining lines depending upon their yield in

crosses with the non-recurrent parent. Those recovered lines which yielded more in crosses than the original  $F_1$  cross were arbitrarily described as high in combining ability. The recovered lines which yielded the same as or less in crosses than the original  $F_1$  cross, but not significantly so, were classified as intermediate in combining ability. The recovered lines which yielded significantly less in crosses than the original  $F_1$  cross as measured at the 5% point were described as low in combining ability.

It will be noted from Table 3 that the differences among the recovered lines for combining ability as measured by their crosses to the non-recurrent parent were great in the recovered lines from C16 and C20. In the case of the recovered lines from C15 and C19 the differences were small and in most cases were not significant at the 5% point.

In general, most of the recovered lines showed a marked improvement in vigor, plant type, and yield over the original lines as observed during the period of selfing following the back pollinations. All the selected recovered lines were definitely superior to the original lines in soundness of the ear. It seemed from these observations that with convergent improvement very definite improvements in vigor characters over the original parental lines can be made in the recovered lines.

#### SINGLE CROSSES FROM SELECTED RECOVERED LINES

In both groups of single crosses some of the crosses between the recovered lines were improved over the original  $F_1$  cross in yield and other characters. Other single crosses between recovered lines were distinctly inferior in yield to the original  $F_1$  crosses. Some crosses showed improvement in lodging resistance and smut resistance, while others were inferior to the original  $F_1$ . No marked improvement in ear length was observed in the crosses. The parental lines of both single crosses were not widely different and the genes in each which affected ear length were probably very similar.

A frequency distribution of the yields of the crosses between the recovered lines in relation to the yields of the original  $F_1$  crosses is given in Table 4. Yields of the crosses given in Table 4 and later in Table 5 are in a frequency distribution of 0, +1, -1, etc., times the standard error of the difference from the yield of the original  $F_1$  crosses.

In this study about one-fourth of the single crosses between recovered lines were significantly higher yielding and one-fourth were significantly lower yielding than the original  $F_1$  crosses.

Since recovered lines which were high as well as low in yield when crossed with the non-recurrent parent were included in these studies, it is desirable to divide the single crosses into groups depending upon the previous performance of their parental lines when tested in crosses to the non-recurrent parent. In addition the possibility of using this yield of a recovered line tested in crosses to the non-recurrent parent as a means of determining the combining ability of recovered lines may be shown. The frequency distribution is given in Table 5.

TABLE 4.—Frequency distribution of yields of single crosses between recovered lines.

Total crosses	Pedigree	Yield in relation to the original F <sub>1</sub> cross								Mean yield of all crosses	Yield of original F <sub>1</sub> cross	
		Class centers of plus 4 and minus 3 times the S.E. of a difference*										Mean class
		-3	-2	-1	0	+1	+2	+3	+4			
8	Rec. C16× Rec. C20	1	4	1	1	—	1	—	—	-1.25	56.0	58.9±1.4
9	Rec. C15× Rec. C19	—	—	4	1	1	1	—	2	+0.78	66.1	64.8±1.4

\*S.E. of a difference = 1.94 bushels per acre.

Although the number of crosses tested in each group was few, it seems that the chances of obtaining single crosses between recovered lines which were higher yielding than the original F<sub>1</sub> crosses were good when both the parental lines were high or one was high and one was intermediate in yield when crossed with the non-recurrent parent. No single crosses yielded more than the original F<sub>1</sub> cross when one of the parental lines was low or both were intermediate or low in yield when crossed with the non-recurrent parent.

The method of determining the value of recovered lines in single crosses by measuring their yield in crosses to the non-recurrent parent seems to have some value.

In this study the yielding ability of the recovered lines when crossed to the recurrent parent did not seem to have a very noticeable relationship to their performance in single crosses.

TABLE 5.—Frequency distribution of yield of single crosses between recovered lines classified into high, intermediate, and low combining lines on the basis of their yield when crossed to the non-recurrent parent.

Classification of recovered lines	Total crosses	Yield in relation to the original F <sub>1</sub> cross								Mean class
		Class centers of plus 4 and minus 3 times the S.E. of a difference*								
		-3	-2	-1	0	+1	+2	+3		
High×high..	6	—	—	2	1	1	—	—	2	+1.17
High×int. . .	5	—	1	2	—	—	2	—	—	0.00
High×low ..	3	—	2	1	—	—	—	—	—	-1.67
Int.×int. . . .	1	—	—	—	1	—	—	—	—	0.00
Int.×low . . .	1	—	1	—	—	—	—	—	—	-2.00
Low×low . . .	1	1	—	—	—	—	—	—	—	-3.00
	17									

\*S.E. of a difference = 1.94 bu. per acre.

From these studies it seems that the first selection of recovered lines may be made by testing them in single crosses to the non-recurrent parent. Those that give low yields in these crosses can be discarded without fear of discarding valuable lines. Then, the higher yielding recovered lines which are also improved in vigor must be tested in all possible combinations in order to determine the most satisfactory single cross. The results also show that some of these single crosses between the selected recovered lines may be expected to yield less than the original  $F_1$  cross.

### SUMMARY

1. It was possible by convergent improvement to make a marked improvement in the recovered lines in vigor, in resistance to smut and lodging, and in soundness of the ear in those instances where the original lines were lacking in these characters.

2. The majority of the recovered lines yielded significantly higher in crosses to the recurrent parent than the original recurrent parent. It seems that a portion of the genotype of the non-recurrent parent was retained with ease by selection in the backcross progenies in 2- and 3-year backcrosses and in the one 4-year backcross.

3. Some recovered lines yielded as well as the original  $F_1$  cross when tested in crosses with the non-recurrent parent. It seems that a number of the lines in these backcrosses had recovered sufficient amounts of the recurrent parent genotype to combine well with the non-recurrent parent.

4. No relationship was found between the performance of the recovered lines when crossed to the recurrent parent and their performance when crossed to the non-recurrent parent.

5. In this study there was no apparent difference in the performance of the recovered lines from 2-year, 3-year, and in the one instance the 4-year backcrosses. From their appearance the recovered lines seemed to approach the appearance of the recurrent parent rather rapidly. It appears that two or three generations of backcrossing are enough to recover a sufficient amount of the genotype of the recurrent parent. One group of 4-year backcrosses used in this study did not seem different from the 2- and 3-year backcrosses in that same line.

6. It was possible to obtain single crosses between recovered lines which yielded significantly higher than the original  $F_1$  cross. The chances of obtaining high-yielding crosses was greatest when the recovered parental lines had been high or intermediate in combining ability as measured by their yield in crosses to the non-recurrent parent.

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# NUT GRASS ERADICATION STUDIES: III. THE CONTROL OF NUT GRASS, *CYPERUS ROTUNDUS* L., ON SEVERAL SOIL TYPES BY TILLAGE<sup>1</sup>

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NUT grass, *Cyperus rotundus* L., is one of the most noxious perennial weeds of cultivated lands in the southern states. It is particularly objectionable in cotton fields. It competes with the cotton seedling for moisture and nutrients and "choppers" destroy many young cotton plants in trying to eliminate nut grass; both tend to break the stand of cotton. It is a serious pest also in gardens, truck crops, nurseries, and flower gardens.

Although the noxious nature of nut grass is recognized wherever it grows, relatively few workers have developed practicable methods for its control. Ranade and Burns (3)<sup>3</sup> found that it could be eradicated in India by plowing during two successive "hot" seasons. Andrews (1, 2), working in the Anglo-Egyptian Sudan, recently reported the eradication of nut grass by a single tillage operation early in the dry season. The latter used a cultivator having overlapping tine-points that cut deep enough to sever the root systems of the deepest tubers. Both India and the Sudan have a long dry season and the pest is killed during this season by the desiccating effect of the hot, dry, tilled soil.

Despite the lack of well-differentiated dry and rainy seasons in the southeastern states, Smith and Mayton (4) at the Alabama Agricultural Experiment Station were able to eradicate nut grass completely, or nearly so, from Norfolk sandy loam soil by plowing at intervals of three weeks during two successive growing seasons. Since nut grass occurs in almost every county of Alabama and infests soil types varying from sand to the heaviest clay, it soon became evident that this method should be tested on soils of various textures before being recommended generally. Consequently, in 1937, cooperative tillage experiments were located with 11 farmers who had soils of the desired types infested with nut grass. The results of these experiments are reported in this paper.

## EXPERIMENTAL METHODS

Eleven experiments were located on soils of the desired types infested with nut grass and farmed by men who had been recommended by county agents. One experiment was located on each of the following soil types: A deep phase of Norfolk sandy loam, Orangeburg fine sandy loam, Ruston fine sandy loam, Amite fine sandy loam, Susquehanna fine sandy loam, Susquehanna very fine

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 159.

sandy loam, Colbert silt loam, and Cecil clay loam. Two experiments were established on Eutaw Clay.

Two treatments were included in all experiments. These consisted of fallowing one plot by plowing with a turn plow at intervals of two weeks during two successive growing seasons and another at intervals of three weeks. Wherever the infested area was large enough and the equipment owned by the cooperating farmer permitted, additional plots were broken at intervals of four weeks; plowed with a scrape<sup>4</sup> at intervals of two weeks; and disked at intervals of two, three, and four weeks during two successive growing seasons. An untilled check plot was included in most of the experiments. In a few experiments where the infested area was small or where the cooperator objected to leaving an area untilled for three years, the check plot was omitted. In these instances some adjacent infested area was observed as a check. The standard plot was 20 by 109 feet, although a few were larger.

The tillage operations were conducted during the 1938 and 1939 growing seasons for nut grass, beginning in April and continuing through October. A few days prior to each plowing date, a double postal card was mailed to each co-operating farmer, indicating the tillage date and the plots to be tilled. After compliance, the cooperator returned the card to the Experiment Station. The tillage operations were delayed occasionally by rain. During the two years, the delays for all cooperators were distributed as follows: 13 of 2 days' duration, 12 of 3, 3 of 4, 3 of 5, 5 of 6, 6 of 7, and 11 of longer than one week, including four times when the tillage operations were omitted entirely.

Infestation records on each plot were taken by the project leaders at the beginning and end of the 1938 and 1939 growing seasons. Two by two foot areas were dug at pre-determined locations on the plots before the 1938 tillage operations were commenced. The tubers were sifted from the soil that was removed from these areas and the living and dead tubers were separated and counted. Adjacent 2 by 2 foot areas were dug in the fall of 1938 and the spring and fall of 1939. At least two areas were dug in each plot at each sampling.

Many of the diggings at the end of the 1939 growing season yielded numerous dead tubers but no live ones. Each of these diggings was proximate to others from which live tubers had been obtained previously. Although these results indicated that nut grass had been eradicated from many plots, it was believed that more reliable conclusions could be drawn if the final infestation records were not made until some time after the tillage operations had been discontinued, since the sample areas were small in size and few in number. Consequently, the plots were not tilled in 1940 so that any live tubers might germinate. The final infestation records, which were taken in August, included tuber counts from the central 10 by 10 foot area of each plot and sprout counts or observations from the whole plot. The number of viable tubers obtained from these larger areas, plus a record of the nut grass sprouts on the plots, formed a reliable index of the efficacy of the different treatments on various soil types.

## RESULTS AND DISCUSSION

The results are presented in Table 1. With very few exceptions, the results obtained from the same tillage treatment on various soil types were comparable, regardless of the differences in soil texture. The

<sup>4</sup>A scrape or sweep is a V-shaped bottom for a one-horse plow normally used in cultivation by southern farmers.

outstanding exception will be disposed of before the more uniform results are discussed.

One of the two experiments on Eutaw clay was located on a low-lying area where the soil remained too wet for long periods of time to permit effective plowing. Although the infestation was markedly reduced by turning at intervals of two and three weeks during two successive growing seasons, the residual infestation at the end of the experiment was too great for the results to be considered satisfactory. Since the results obtained on the other Eutaw clay site were similar to those from other soil types, it is evident that the terrain was more responsible than the soil texture for the unsatisfactory results. No tabular data are presented for this experiment and the discussion that follows does not pertain to it.

In general, tuber counts indicated that the infestation had been reduced 90% or more by turning at intervals of two and three weeks, respectively, during a single growing season. The lone exception was the three-week plot on Cecil clay loam where the tuber count indicated a reduction of about 50%. The results for the two-week plot, however, compared favorably with those for other soil types. The results for the four-week intervals were not so uniform as for the shorter intervals.

Tuber counts at the end of the second consecutive tillage season indicated complete or nearly complete eradication of nut grass by turning at intervals of two and three weeks, respectively, on all soil types and at intervals of four weeks on the soil types on which this treatment was used. As has been pointed out previously, these records did not constitute the final test of the efficacy of the various treatments. The final infestation records were made in August, 1940, after the plots had remained untilled for a growing season.

The most reliable records would have been obtained if the whole of each plot had been dug to the level of the deepest tuber and all of the dead and living tubers had been removed and counted. The time required and the cost of labor made such a procedure impracticable. The final records, therefore, were obtained by removing, sorting living from dead, and counting the tubers from the central 10 by 10 foot area of each plot, and by examining each plot for nut grass sprouts, except the plots on Orangeburg and the check plot on Susquehanna fine sandy loam. (See last two footnotes to Table 1.)

The final tuber counts, representing a large portion of each plot, confirmed the tuber counts made at the end of the second tillage season in indicating that turning the various soils at all intervals almost or completely eradicated nut grass. None of the two-week, two of the three-week, and one of the four-week plots yielded live tubers at the final digging. It is significant that the results from turning plastic soils, such as Eutaw and Susquehanna, were as satisfactory as those from plowing sandy soils such as Norfolk and Ruston.

Actually, complete eradication of nut grass was effected on very few plots. Careful observations showed a few nut grass sprouts on most of the turned plots, the largest numbers being on the plots turned at intervals of four weeks. These observations do not discredit tuber



TABLE 1.—*Relation of frequency of tillage to eradication of nut grass on various soil types.*

Tillage*	Number of living and dead tubers†									
	1938		1939				1940		Living	Dead
	Fall		Spring		Fall					
	Living	Dead	Living	Dead	Living	Dead				
Norfolk Sandy Loam										
Plowed every 2 weeks.....	213	12	296	7	249	0	146	0	1,096	
Plowed every 3 weeks.....	133	2	189	1	254	0	95	0	945	
Plowed every 4 weeks.....	101	54	277	7	200	1	95	0	1,046	
Plowed with a scrape every 2 weeks.....	44	3	178	0	109	0	46	0	752	
No tillage†.....	39	39	111	8	81	7	41	6	1,004	
Orangeburg Fine Sandy Loam§										
Plowed every 2 weeks.....	208	1	66	1	3	0	30	—	—	
Plowed every 3 weeks.....	38	2	25	3	6	0	15	—	—	
Disked every 2 weeks.....	205	3	172	2	9	1	14	—	—	
Plowed with scrape every 2 weeks.....	144	10	63	5	21	0	7	—	—	
Ruston Sandy Loam										
Plowed every 2 weeks.....	116	2	52	2	38	0	19	0	192	
Plowed every 3 weeks.....	69	1	46	3	60	0	49	0	451	
Plowed every 4 weeks.....	48	3	62	2	48	0	28	0	1,251	
Disked every 2 weeks.....	68	13	40	4	71	1	48	3	227	
Disked every 3 weeks.....	33	18	37	15	47	10	17	117	417	
Disked every 4 weeks.....	94	18	60	20	51	5	31	161	845	
Plowed with scrape every 2 weeks.....	4	1	62	1	37	0	26	0	144	
No tillage.....	84	89	27	86	16	181	29	361	2,625	

## Red Bay Fine Sandy Loam

Plowed every 2 weeks.....	189	5	266	5	54	0	12	0	125
Plowed every 3 weeks.....	510	14	170	68	53	0	38	0	85
Plowed every 4 weeks.....	118	29	315	52	68	1	27	0	310
No tillage.....	92	148	28	114	44	379	70	1,790	990

## Amite Fine Sandy Loam

Plowed every 2 weeks.....	412	20	395	2	239	1	121	0	1,823
Plowed every 3 weeks.....	505	13	317	3	239	1	50	0	780
Plowed every 4 weeks.....	330	13	305	19	193	0	116	0	916
Disked every 2 weeks.....	265	9	342	0	247	5	146	0	625
Disked every 3 weeks.....	378	7	205	2	248	0	98	0	632
Disked every 4 weeks.....	367	12	245	10	116	0	51	0	375
Plowed with scrape every 2 weeks.....	418	16	376	1	184	1	60	0	218
No tillage.....	438	564	124	466	87	547	32	2,254	6,324

## Susquehanna Fine Sandy Loam

Plowed every 2 weeks.....	58	2	95	0	76	0	30	0	516
Plowed every 3 weeks.....	62	1	48	1	38	0	39	0	394
Plowed every 4 weeks.....	75	3	111	2	68	7	92	0	957
Plowed with scrape every 2 weeks.....	42	0	54	0	34	0	47	0	179
No tillage  .....	20	37	35	38	37	39	20	—	—

## Susquehanna Very Fine Sandy Loam

Plowed every 2 weeks.....	216	11	88	6	25	0	6	0	208
Plowed every 3 weeks.....	202	23	168	28	75	4	34	0	440
Plowed every 4 weeks.....	410	203	234	119	155	19	108	3	858
Plowed with scrape every 2 weeks.....	122	71	114	28	35	2	15	0	471
No tillage.....	294	419	56	289	22	473	16	3,541	16,575

\*Tillage commenced April 1 in 1938 and 1939 and continued through October each year.

†Each figure for 1938 and 1939 represents the average number of tubers from two 2 by 2 foot diggings. The figures for 1940 represent the number of tubers from the central 10 by 10 foot area of the plots in August.

‡The original infestation was recorded as "very spotted". Although adjacent to the other plots, this plot was situated on the crest of a dry ridge.

§Tillage channel opened into these plots during the winter of 1939 and no infestation records were obtained in 1940.

||Plot destroyed by the State Highway Department during the winter of 1939.

TABLE I.—*Continued.*

Tillage*	Number of living and dead tubers†									
	1938		1939				1940			
	Fall		Spring		Fall					
	Living	Dead	Living	Dead	Living	Dead	Living	Dead	Living	Dead
Colbert Silt Loam										
Plowed every 2 weeks.....	346	1	35	3	38	1	18	0	88	
Plowed every 3 weeks.....	974	46	417	29	239	16	126	0	422	
Cecil Clay Loam										
Plowed every 2 weeks.....	132	3	98	1	41	0	33	0	390	
Plowed every 3 weeks.....	111	56	380	13	170	0.5	76	6	885	
Plowed every 4 weeks.....	65	6	64	1	44	1	27	0	385	
Plowed with scrape every 2 weeks.....	80	—	177	0	84	2	82	0	605	
No tillage.....	38	44	12	55	7	90	3	1,880	1,400	
Butaw Clay										
Plowed every 2 weeks.....	234	16	191	16	117	0	53	0	84	
Plowed every 3 weeks.....	474	38	318	7	537	0	132	2	305	
Plowed every 4 weeks.....	357	30	28	1	238	0	107	0	93	
Plowed with scrape every 2 weeks.....	89	3	210	5	72	0	118	2	168	
No tillage.....	112	146	128	238	98	124	57	45	526	

\*Tillage commenced April 1 in 1938 and 1939 and continued through October each year.

†Each figure for 1938 and 1939 represents the average number of tubers from two 2 by 2 foot diggings. The figures for 1940 represent the number of tubers from the central 10 by 10 foot area of the plots in August.

counts as one method of measuring nut grass infestation; they merely show that plowing at intervals of two, three, and four weeks, respectively, during two growing seasons reduces the population of viable tubers to such an extent that the whole plot must be used as a sample if a complete record is to be obtained.

Many of the sprouts were dug carefully and found to have their origins in tubers located in the subsoil. This fact suggested the desirability of knowing the maximum depth at which the tubers are formed in the various soil types, and the distribution of tubers at various depths. Consequently, depth distribution studies were made on seven of the areas where tillage experiments were located. The soil from a 2 by 2 foot quadrat was removed by 2-inch layers and tubers from each layer were counted separately (Table 2). These data show that a large percentage of the tubers in all soil types were situated in the upper 6 inches of soil, the percentage varying from 84 in Eutaw clay to 99 in Cecil clay loam. No live tubers were found below the 12 to 14 inch depth in any soil except Orangeburg fine sandy loam and none below 16 inches in that soil.

TABLE 2.—*Depth distribution of live tubers in several soil types.*

Soil type	Number of live tubers in a 2 by 2 foot area at depths indicated in inches								
	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18
Orangeburg fine sandy loam.....	96	145	68	18	17*	—*	6	6	0
Ruston fine sandy loam.....	40	92	53	10	1	1	0	0	0
Amite fine sandy loam..	240	166	165	58	7	1	3	0	0
Susquehanna fine sandy loam.....	270	304	95	18	51	6	1	0	0
Colbert silt loam.....	222	155	31	4	1	0	0	0	0
Cecil clay loam.....	195	100	58	20	5	6	4	0	0
Eutaw clay.....	100	143	162	57	9	6	0	0	0

\*Through error, tubers from the 8-10 and 10-12 inch layers were combined.

An explanation for the near, but rarely complete, eradication of nut grass by tillage operations is found in the depth distribution of tubers. Tubers in the surface soil are isolated from the deeper tubers and roots by repeated tillage and subjected to desiccation and starvation. Those below plow depth usually are not subject to desiccation, but may be starved gradually by repeated removal of the aerial portions of the plant. Apparently, all tubers within plow depth on all soil types were killed by plowing at intervals of three weeks or less during two successive growing seasons, but an occasional tuber below plow-depth survived. The efficiency of tillage as a means of destroying nut grass, therefore, is based on the fact that most of the tubers are located in the surface soil.

Plots on Amite fine sandy loam and Ruston fine sandy loam were disked at intervals of two, three, and four weeks, respectively, during two successive growing seasons and at intervals of two weeks on Orangeburg fine sandy loam. A tractor-drawn disc was used on the

Amite and Orangeburg plots, but a light, horse-drawn disc was used on the Ruston plots. The tractor-drawn disc was as effective as the turn plow in destroying nut grass, but the light disc was not so effective. If a disc is to be used for controlling nut grass, it must be a heavy one.

The results from plowing with a scrape at intervals of two weeks during two successive growing seasons were comparable to those from plowing with a turn plow at the same intervals on the following soil types: Norfolk sandy loam, Orangeburg fine sandy loam, Ruston fine sandy loam, Amite fine sandy loam, Susquehanna fine sandy loam, Susquehanna very fine sandy loam, Cecil clay loam, and Eutaw clay. It is interesting to note that on these plots equipment was used that is available on the smallest farming unit.

### SUMMARY AND CONCLUSIONS

Eleven cooperative tillage experiments dealing with the eradication of nut grass were conducted on 10 soil types during the growing seasons of 1938 and 1939. The soil types varied from a deep phase of Norfolk sandy loam to Susquehanna very fine sandy loam (having a shallow surface soil and a plastic clay subsoil) and Eutaw clay.

Near eradication was obtained by certain tillage treatments on all soil types. Based on tuber counts alone, plowing at intervals of four weeks gave as good results as turning at shorter intervals. When the sprout counts of the final infestation records were taken into consideration, however, it was obvious that plowing at intervals of two or three weeks was more effective.

Plowing with a scrape gave results comparable to those obtained with a turn plow. Thus, equipment available on the smallest farming unit can be used successfully to combat nut grass.

The disking experiments emphasized the necessity of a suitable disc if this implement is to be used for the control of nut grass. A light, horse-drawn disc was unsatisfactory, but a tractor disc was comparable to a turn plow.

The only experiment in which satisfactory control of nut grass was not obtained was one situated on a low, poorly drained area of Eutaw clay. The results indicate that tillage methods should not be used for nut grass control on areas that are likely to remain wet for long periods.

The destruction of nut grass by tillage is based on the fact that most of the tubers are located in the upper 6 inches of soil. Tubers isolated by repeated plowing are killed by desiccation and starvation. An occasional tuber below plow depth may live through two successive tillage seasons. These tubers are a source of potential reinfestation. It is believed, however, that any farmer who will plow at regular intervals for two growing seasons to destroy nut grass will complete the eradication of the pest by digging up each tuber as it sprouts the following year.

It is concluded that nut grass can be nearly eradicated from soils varying in texture from sandy loam to plastic clay by plowing at intervals of three weeks during two successive growing seasons. If a

clean-cultivated crop is planted the following year and if the few remaining tubers are removed as they sprout, the eradication will be complete.

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INHERITANCE OF GROWTH HABIT, COTYLEDON COLOR,  
AND CUP-LEAF IN *MELILOTUS ALBA*<sup>1</sup>E. E. HARTWIG<sup>2</sup>

SINCE improvement of sweetclover is of relatively recent origin, only limited studies have been made on the inheritance of its characteristics. In addition to contributing to breeding, the genetics of sweetclover is of value for making determinations on the isolation requirements for maintaining the purity of improved varieties. In this paper the inheritance of three characters, namely, growth habit, cotyledon color, and cup-leaf, is discussed.

## GROWTH HABIT

Considerable interest has been focused upon the inheritance of growth habit in biennial white blossom sweetclover since the Alpha type was first described, particularly because of the forage value of leafy, fine-stemmed types. The Alpha variety is short-growing and has fine stems and a high proportion of leaves to stems. Stevenson (6)<sup>3</sup> has used the term "dwarf branching" to describe this type. Kirk (5) and Stevenson (6) have studied the inheritance of the Alpha type and found it to be a simple recessive to the common growth type with the F<sub>2</sub> generation giving a close fit to a 3:1 ratio. Clarke (1) studied the inheritance of a similar low-growing type which he termed "dwarf-bunch." The dwarf-bunch also behaves as a simple recessive to the common growth type. In the cross Alpha × dwarf-bunch, the F<sub>1</sub> was of the common growth type. The F<sub>2</sub> segregation was interpreted as a 9:6:1 ratio in which the double recessive was lethal, thus giving nine tall to six dwarf-type plants. The two recessive genes involved were named bunched dwarf (*bd*) and spreading dwarf (*sd*), respectively.

Two other leafy, short, fine-stemmed types have been grown in the Urbana nursery. These are F. C. 13074 and a segregate from F.P.I. 89911. F. C. 13074 was obtained from Matthew Fowlds, South Dakota State College, Brookings, S. D. F.P.I. 89911 was introduced from Spain by the U. S. Dept. of Agriculture. One plant of F.P.I. 89911, selected in the summer of 1937, produced common and small type plants in the first selfed generation progeny. Although both of these low-growing types differ somewhat from the Alpha type, yet,

<sup>1</sup>Contribution from the Department of Agronomy, Illinois Agricultural Experiment Station, Urbana, Ill., in cooperation with the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture. Part of a thesis submitted in partial fulfillment of the Ph. D. degree in agronomy, University of Illinois. Published with the approval of the Director of the Station. Received for publication September 9, 1941.

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 166.

for convenience, the term "dwarf-branching" will be used to describe them.

In the cross F. C. 13074  $\times$  common type, the  $F_1$  plants were of the common growth type. Two  $F_2$  progenies were planted in the 1939-40 field nursery and two additional  $F_2$  progenies in the 1940-41 field nursery. All plants were classified in the first year's growth. The agreement with expectation of these four  $F_2$  progenies was tested individually and collectively by means of the goodness of fit test (Table 1).

TABLE 1.—Numbers of common type and dwarf-branching plants in four segregating  $F_2$  progenies and values of  $X^2$  when tested for goodness of fit to a 3:1 ratio.

Total	Common type	Dwarf-branching	$X^2$
16.....	9	7	3.000
58.....	41	17	0.574
39.....	32	7	1.034
91.....	68	23	0.004
204.....	150	54	4.612

Degrees of freedom 4.  
P between .30 and .50.

When tested individually, each of the four  $F_2$  families has a  $X^2$  value of less than 3.841, which is the .05 point with 1 degree of freedom. A value for P of between .30 and .50 was obtained when the four  $X^2$  values were considered collectively. These results indicate therefore that the growth type of F. C. 13074 is a simple recessive to the common growth type.

As stated, the  $S_1$  progeny of a plant selection from F.P.I. 89911 segregated for growth habit. A total of 66 plants was grown, 50 of these being of the common growth type and 16 of the dwarf-branching type. This segregation is a very close approximation to a 3:1 ratio and indicates that here, too, the dwarf-branching character is dependent upon a single gene difference.

In the winter of 1939-40 reciprocal crosses were made in the greenhouse between F. C. 13074 and Alpha, and F. C. 13074 was crossed with the dwarf-branching type from F.P.I. 89911. In each case the  $F_1$  plants were of the common growth type. A few of these  $F_1$  plants were grown in the greenhouse in the spring of 1940 and given continuous light to induce flowering. A single  $F_1$  plant was grown from each of the crosses, Alpha  $\times$  F. C. 13074 and F. C. 13074  $\times$  F.P.I. 89911. Honey bees were used to aid pollination. Three plants were grown of the cross F. C. 13074  $\times$  Alpha and these were sib-pollinated with the aid of honey bees. The  $F_2$  populations were started in the greenhouse early in June and later transplanted to the field nursery. Plants were classified in the fall of 1940 on the basis of their first year growth characters.

In the  $F_2$  population of the crosses Alpha  $\times$  F. C. 13074, 26 plants were of the common growth type and 18 were of the dwarf-branching type. When this phenotypic ratio was tested for goodness of fit to a 9:7 ratio, a  $X^2$  of 0.140 was obtained. The reciprocal cross gave 74



plants of the common growth type and 61 of the dwarf-branching habit. On the basis of a 9:7 ratio a  $X^2$  of 0.113 was obtained. For 1 degree of freedom the corresponding P value for each of these  $X^2$  values is between .70 and .80. Hence, the agreement of observed with expected on the basis of a 9:7 ratio is very good and indicates that different genes are responsible for the dwarf-branching character in Alpha and in F. C. 13074.

The  $F_2$  population of the cross F. C. 13074  $\times$  F.P.I. 89911 contained 22 plants of the common growth habit and 11 of the dwarf-branching type. On the basis of a 9:7 ratio a  $X^2$  value of 1.46 was obtained. For 1 degree of freedom the corresponding P value is between .20 and .30. These results along with the fact that the  $F_1$  was of the common growth type show that the genes determining the dwarf-branching character in F. C. 13074 and in F.P.I. 89911 are not identical.

The results obtained from these crosses along with those obtained by Clarke suggest that several non-allelomorphic genes are responsible for the dwarf-branching character in common biennial white blossom sweetclover. Data are presented showing that different genes determine the dwarf-branching character in Alpha and F. C. 13074 and in F. C. 13074 and F.P.I. 89911. Since Alpha and F.P.I. 89911 differ somewhat in growth habit though both are dwarf-like, it is tentatively assumed that these two types also differ genetically. For the same reason, it is assumed that neither F. C. 13074 nor F.P.I. 89911 is identical with Clarke's bunch dwarf type. It is suggested that the different dwarf-branching types be symbolized as follows: Alpha or spreading dwarf,  $d_1$ ; bunched dwarf,  $d_2$ ; F. C. 13074,  $d_3$ ; and F.P.I. 89911,  $d_4$ .

#### COTYLEDON COLOR

In the growth habit study just described, F. C. 13074 produced seed with green cotyledons as contrasted to the yellow cotyledons found in common sweetclover. In 1937, selfed seed was harvested from four  $F_1$  plants which were natural crosses between the dwarf-branching type having green cotyledons and the tall type having yellow cotyledons. These  $F_1$  plants could be easily recognized because of their tall growth habit. The  $F_2$  segregation for cotyledon color on these plants gave a close fit to a 3:1 ratio with yellow cotyledon dominant to green.<sup>4</sup>  $F_3$  data substantiated the hypothesis that a single recessive gene was responsible for the green cotyledon character. From a population of 106  $F_2$  plants, 25 produced only green cotyledon seeds, 57 produced seed segregating for cotyledon color, and 24 produced only yellow cotyledon seeds. However, in contrast to the results obtained from the  $F_2$  and  $F_3$  populations, reciprocal crosses gave evidence that cotyledon color was maternally inherited in the  $F_1$ . These results were discussed in an unpublished Master's thesis (4). Fowlds (3) also found that green cotyledon is a simple recessive to yellow cotyledon but considered seed coat color to be involved.

<sup>4</sup>Segregation for cotyledon color was distinct. All seeds had yellow seed coats which were sufficiently transparent to permit accurate classification for cotyledon color.

Several reciprocal crosses have been made between types having green and yellow cotyledon, and in every case the  $F_1$  seed has resembled the seed of the maternal parent. With yellow cotyledon dominant to green, the  $F_1$  seed even though borne on the green parent would be expected to have yellow cotyledons. The behavior of the  $F_1$  might lead one to suspect that the color difference is in the seed coat and not in the cotyledons. However, this is not the case as  $F_1$  plants produce seed showing clear segregation for cotyledon color and  $F_2$  plants producing  $F_3$  cotyledons have no indication of segregation in seed coat color.

Open-pollinated seed was harvested from plants of F. C. 13074 in 1937, 1938, and 1939, when these plants were grown in close proximity to tall-growing, yellow cotyledon plants. This seed appeared to have green cotyledons like the selfed seed. However, upon growing plants from the open-pollinated seed, it was quite apparent that crossing had occurred with the tall-growing type with yellow cotyledons.

Karl F. Manke, while with the Division of Forage Crops and Diseases of the U. S. Dept. of Agriculture working in cooperation with the Nebraska Agricultural Experiment Station, suggested that under Nebraska conditions the seeds produced on the green cotyledon plant following cross-pollination with the yellow cotyledon type were more yellowish in appearance than the selfed seeds produced upon the same plant. As a check on this suggestion, open-pollinated seed was obtained from 10 dwarf-branching plants grown at Lincoln, Nebraska, which had produced only seed with green cotyledons upon selfing but which had been grown near tall plants having yellow cotyledons. These seeds were classified and a planting made in the 1940 field nursery at Urbana, Illinois, giving 10 plants from yellowish seed and 10 from green seed from each of the 10 mother plants. It was possible to classify the progeny in the first year growth, since the green cotyledon seeds, if they were selfs or sibs, should produce only plants of the dwarf-branching type, and the yellowish seeds, if they were outcrosses, should produce only tall plants. Plant observations showed that of the 100 plants grown from seed classified as green, 5 were tall and therefore outcrosses, and of the 100 plants grown from seed classified as yellowish, 21 were of the dwarf-branching type and therefore either selfs or sibs. These results indicate that under Nebraska conditions a higher percentage of the outcross seeds can be recognized, thus giving evidence that the dominance of yellow cotyledon expresses itself to a greater extent in the  $F_1$  cotyledon under Nebraska than under Illinois conditions. In discussing the inheritance of green seed color, Fowlds (3) implies that his  $F_1$  plant was grown from a green cotyledonous seed. If that were the case, his results would be in agreement with the findings of this investigation.

On the basis of  $F_2$  and  $F_3$  results, the same genetic explanation of cotyledon color can be given for sweetclover as was described by White (7) for the garden pea.  $Y$  and  $G$  are genes for yellow and green pigments, respectively. Both of these genes are assumed to be present in all plants observed. The gene  $I$  causes green pigment to fade at maturity and is the gene found to be segregating in sweetclover. Apparently  $I$  cannot cause the green pigment to fade at maturity

when acting in the cytoplasm of the *ii* parent. However, certain environmental conditions, as in Nebraska, may permit *I* to operate to a limited extent.

#### CUP-LEAF

In the winter of 1937-38 a first generation selfed population of four plants from seed of F. C. 13074-3 was grown in the greenhouse. One of these plants was observed to have an abnormal leaf which has been designated "cup" (Fig. 1). A population of 50  $S_1$  plants was grown in the 1938-39 field nursery. Of these, 33 plants had normal



FIG. 1.—Cup (left) and normal (right) leaves of sweetclover.

leaves and 17 had cup leaves. The plants classified as cup also had some normal leaves, but a high proportion of the leaves was cupped.

The cup-leaved plant grown in the greenhouse in the winter of 1937-38 was crossed with a plant having normal leaves. The  $F_1$  plant produced normal leaves. An  $F_2$  population of 92 plants was grown in the 1940-41 field nursery. A ratio of 71 normal leaved to 21 cup-leaved plants was observed, a deviation of only 2 from expectation on the basis of a 3:1 ratio.

Two segregating  $F_3$  progenies were also grown in the 1940-41 field nursery and ratios of 13:1 and 31:8 were observed. Each progeny was tested for goodness of fit to a 3:1 ratio and  $X^2$  values of 2.38 and 0.42 were obtained, indicating a satisfactory fit. These data indicate that in the inheritance of cup-leaf a single pair of genes,  $Cc$ , is involved.

Delwiche and Renard (2) have reported finding a cup- or pitcher-leaved character in peas. The cup character was inherited as a simple recessive; however, all cup-leaved plants were sterile.

#### TEST FOR LINKAGE

In the cross  $F. C. 13074-3 (iid_3d_3cc) \times$  the common type ( $IID_3D_3CC$ ), the  $F_1$  plant was of the common growth type with normal leaves and produced selfed seeds segregating for cotyledon color—256 yellow to 91 green, a very good fit to a 3:1 ratio.

An  $F_2$  population of 92 plants, made up of 46 plants from yellow cotyledon seeds and 46 plants from green cotyledon seeds, was grown in the 1940 field nursery. Table 2 gives the distribution of plants in the various classes and the numbers expected with independent inheritance.

TABLE 2.— $F_2$  distribution of plants grown from yellow and green cotyledonous seeds which segregated for leaf character and plant type along with numbers expected with independent inheritance.

Cotyledon color	Common growth type		Dwarf branching type	
	Normal leaf	Cup leaf	Normal leaf	Cup leaf
Yellow.....	26	8	10	2
Green.....	26	8	9	3
Total.....	52	16	19	5
Expected.....	52	17	17	6

The agreement of numbers observed to numbers expected on the basis of independent inheritance is very close; hence, it may be concluded that the genes  $i$ ,  $d_3$ , and  $c$  are independent.

#### SUMMARY

Genetic studies with *Melilotus alba* have shown that:

1. The growth habits of  $F. C. 13074$  and of the dwarf-branching segregate of  $F.P.I. 89911$  are inherited as simple recessives to the common growth type.

2. The gene determining the dwarf-branching character in F. C. 13074 is different from the gene determining a similar character in the Alpha type and also different from the gene determining the dwarf-branching type in F.P.I. 89911. These genes are designated as  $d_1$  for Alpha,  $d_3$  for F. C. 13074, and  $d_4$  for F.P.I. 89911.

3. Yellow cotyledon color is a simple dominant to green cotyledon, but the  $F_1$  cotyledon color usually resembles that of the maternal parent. Environment appears to play a part in determining the color of the  $F_1$  cotyledon.

4. Cup-leaf is determined by a single gene which is recessive to normal leaf.

5. Genes  $i$  (green cotyledon),  $c$  (cup-leaf), and  $d_3$  (growth habit of F. C. 13074) are independently inherited.

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# CHEMICAL COMPOSITION AND GRAZING VALUE OF NAPIER GRASS, *Pennisetum purpureum* SCHUM., GROWN UNDER A GRAZING MANAGEMENT PRACTICE<sup>1</sup>

R. E. BLASER, W. G. KIRK, AND W. E. STOKES<sup>2</sup>

NAPIER grass, *Pennisetum purpureum* Schum., is a robust, cane-like (non-saccharine), leafy perennial which grows 7 to 15 feet in height. Thompson (13)<sup>3</sup> reports that Napier grass was introduced into the United States in 1913 and that since 1915 the grass has been distributed in small lots for planting in numerous places in Florida.

Although Napier grass was distributed widely in Florida as early as 1915, until recently it has not reached any great economic value. Factors limiting its economic importance were improper management and the development of a fungous disease known as "eyespot" (*Helminthosporium ocellum* Faris), the latter being reported first by Leukel and Camp (4). The development of eyespot-resistant strains of Napier grass by Ritchey and Stokes (10) will no doubt stimulate the utilization of Napier grass in Florida and in the southeastern part of the United States.

In tropical countries Napier grass is utilized primarily for silage, thus the research work has been devoted primarily to the frequency of cutting as related to yield and chemical composition of silage (3, 8, 9, 15). In Florida research work has been conducted to study the yield and value of Napier grass primarily for silage (2, 6, 12).

Wilsie and Takahashi (14) report successful use of Napier grass by the Princeville Plantation Company's ranch on Kauai as follows: "Here may be found more than 600 acres in pure stands of Napier grass, the paddocks ranging in age from recent plantings to others more than 12 years old. The horses as well as the cattle on the ranch are fed almost entirely on Napier grass and keep in excellent condition. The paddocks are grazed heavily, the cattle being turned in only after the grass has reached a height of six feet or more. After the pasture has been well eaten down, the cattle are put into a paddock which has been resting or recovering."

Results of research conducted by the Florida Experiment Station indicate that Napier grass is a promising grass for grazing, if managed properly.

## EXPERIMENTAL PROCEDURE

### HEAVILY FERTILIZED NAPIER GRASS

A 15-acre area of land, primarily Leon, Plummer, and Alachua sandy soil series, was cleared, plowed, and planted to Napier grass in March 1937. Rhizomes

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 174.

of Napier grass (mass eyespot-resistant selections) were spaced 2 feet and planted 4 to 6 inches deep in rows 8 feet apart. A 5-7-5 fertilizer was applied at the rate of 400 pounds per acre in the row previous to planting. Additional fertilizer consisted of two to five applications of nitrate of soda (75 to 100 pounds per acre) as a side dressing.

Cultural practices included two cultivations in 1937 to control weeds. In subsequent years the grass was disked with a double disk harrow in February or March and cultivated once between the rows in 1938 and 1939.

A crop of silage was cut in June 1937, after which the area was fenced into five fields of 3 acres each to permit rotational grazing. Long-yearling grade Hereford and Brahman steers, averaging approximately 450 pounds when grazing was started, were used each year as test animals. Grazing was initiated when the grass reached a height of approximately 3 feet. A sufficient number of steers was used to consume most of the grass blades in one paddock in 5 to 7 days. Steers were added or withdrawn so that the forage in any one paddock would be consumed in 5 to 7 days. By this method the grass was grazed to heights between 3 and 5 feet. The steers were weighed individually each time they were rotated to a different field. Water and mineral supplement as recommended by Becker, *et al.* (1) and by Neal (5), were available to the animals at all times.

Four movable quadrats 10 feet square were placed in each paddock to protect grass for yield records and chemical analyses. Grass samples were taken immediately after the animals were moved to a rested paddock. The protected grass was plucked by hand to simulate the grazed grass as nearly as possible. All quadrats were relocated after the samples were plucked.

Four panels 5×10 feet, fastened at the corners, were used to enclose the quadrats. Panel frames were made of 2×4 inch lumber over which woven wire was stretched and fastened.

#### LIGHTLY FERTILIZED NAPIER GRASS

A 7½-acre hammock (primarily Alachua soil series) was cleared and planted to Napier grass in 1938. Planting and cultural practices were the same as those already described. An application of 400 pounds of a 5-7-5 fertilizer was made annually. No side dressings of nitrogen were made. Heifers were used as test animals and managed as on the highly fertilized Napier grass.

A small percentage of the grasses consumed by test animals in both experiments consisted of Bermuda grass (*Cynodon dactylon*), *Digitalis* spp., and traces of other plant species.

### RESULTS AND DISCUSSION

#### COMPOSITION OF NAPIER GRASS WHEN MANAGED FOR GRAZING

The growth condition of Napier grass at the time steers were placed into rested paddocks for grazing is shown in Fig. 1. The ungrazed residue of Napier grass just before the removal of the animals to a rested paddock is shown in Fig. 2. Generally, the animals were removed after 75 to 90% of the leaf blades were consumed.

The composition of plucked samples of Napier grass taken to resemble the grass actually consumed by the animals is shown in Table 1. These results show that Napier grass as managed for grazing remained quite uniform in the constituents for which it was analyzed



FIG. 1.—This photograph represents an average growth condition of Napier grass at the time animals were placed in a rested paddock for grazing.

during both seasons. The average composition of various constituents (moisture-free basis) for the 1938 and 1939 seasons are as follows: Protein, 12.3%; fiber, 32.5%; nitrogen-free extract, 45.8%; crude fat, 2.9%; ash, 6.5%; calcium, 0.48%; and phosphorus, 0.33%.

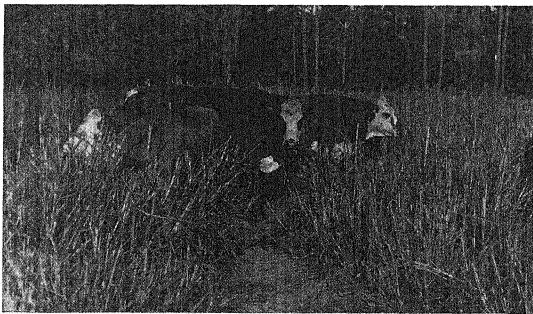


FIG. 2.—This photograph shows the ungrazed residue of Napier grass just before the animals were moved to a rested paddock. The animals were generally removed when 75% to 90% of the leaf blades were consumed. New growth developed rapidly by whirls of leaves stooling from the nodes.



Digestion trials of Napier grass blades conducted by Neal, Kidder, and Rusoff (7) showed this grass to contain 8.4% digestible crude protein and 65.4% total digestible nutrients on dry basis.

Grazed portions of the Napier grass (primarily leaves) averaged 12.3% protein as compared to only 5.5% protein for the ungrazed residue (primarily stems). The ungrazed residue was higher in fiber and lower in fat, ash, phosphorus, and calcium content than the grass consumed by animals. The nitrogen-free extract was similar for the consumed grass and the ungrazed residue.

The dry matter content of the ungrazed residue was 13.8% as compared with 19.8% for the grass consumed. The high proportion of stems in the ungrazed residue accounts for the low dry matter and low protein content.

TABLE I.—*Chemical composition on dry basis of Napier grass consumed by steers and of ungrazed residue, 1938 and 1939.\**

Date sample represented	Dry matter, %	Crude protein, %	Crude fiber, %	N-free extract, %	Crude fat, %	Ash, %	Ca, %	P, %
Napier Grass (Primarily Blades) Grazed by Steers, 1938								
May 3 to May 20.....	—	11.1	32.9	47.1	2.4	6.5	0.47	0.29
May 21 to May 30.....	—	10.0	33.3	47.2	2.6	6.9	0.59	0.26
May 31 to July 13.....	—	12.5	32.0	45.9	3.1	6.5	0.57	0.32
July 14 to Sept. 6.....	—	11.6	33.0	45.8	3.0	6.6	0.43	0.36
Sept. 7 to Oct. 9.....	—	14.0	32.4	44.4	2.8	6.4	0.29	0.39
Oct. 10 to Oct. 18.....	—	13.3	30.8	47.1	2.8	6.0	0.46	0.35
Av., 1938.....	18.9	12.1	32.4	46.2	2.8	6.5	0.47	0.33
Napier Grass (Primarily Blades) Grazed by Steers, 1939								
Apr. 28 to May 10.....	17.3	11.8	31.7	47.0	2.9	6.6	0.56	0.31
May 11 to May 21.....	21.0	10.1	33.1	46.3	2.8	7.7	0.65	0.28
May 22 to May 25.....	22.9	9.9	33.4	48.2	2.9	5.6	0.55	0.31
May 26 to June 22.....	18.0	13.5	33.0	44.0	2.6	6.9	0.46	0.35
June 23 to Aug. 11.....	20.5	11.9	33.6	45.0	3.0	6.5	0.41	0.33
Aug. 10 to Sept. 25.....	22.7	14.5	32.6	43.2	3.0	6.7	0.38	0.36
Sept. 26 to Oct. 11.....	21.1	15.7	30.9	43.9	3.4	6.1	0.39	0.39
Av., 1939.....	20.5	12.5	32.6	45.4	2.9	6.6	0.49	0.33
Av., 1938-39.....	19.8	12.3	32.5	45.8	2.9	6.5	0.48	0.33
Napier Grass Ungrazed Residue (Primarily Stems and Sheaths), 1939								
May 5 to June 1.....	—	5.3	38.6	47.8	1.4	6.9	0.25	0.29
June 23 to July 19.....	—	5.6	38.0	49.5	1.7	5.2	0.24	0.28
July 20 to Sept. 26.....	—	5.5	37.9	49.1	1.8	5.7	0.25	0.31
Av., 1939.....	13.8	5.5	38.2	48.8	1.6	5.9	0.25	0.29

\*Samples taken from the 15-acre Napier grass grazing experiment. The writers gratefully acknowledge the cooperation of W. M. Neal and assistants of the Animal Nutrition Laboratory in making the chemical analysis.

Napier grass as managed for soilage (cut every 6 to 8 weeks) by Joachim and Pandittisekere (3) averaged, on a dry basis, 8.82% crude

protein, 30.97% crude fiber, 45.91% nitrogen-free extract, 1.72% crude fat, and 12.58% ash. The dry matter averaged 16.2%. Wilsie, *et al.* (15), who managed this grass similarly, report analysis as follows: Protein, 7.90%; crude fiber, 28.81%; nitrogen-free extract, 41.85%; crude fat, 2.19%; and ash, 19.24%. Paterson (8) reports similar analyses of Napier grass when cut bi-monthly.

A comparison of these data shows that Napier grass as managed for grazing produced a forage which is higher in dry matter and protein than Napier grass managed for soilage. The chemical analyses of blades for the two management practices are probably comparable, but the inclusion of stems in soilage causes the reduced dry matter and protein content.

The variation in the remaining constituents between Napier grass managed for soilage and grazing, with the exception of ash, may be considered insignificant. The ash content of Napier grass grown for soilage was higher than that of Napier grass produced under grazing management practices. The differences in fertility of soils and soil contamination in samples may be the primary factors influencing the ash content.

#### GRAZING TESTS

The average gains of steers and steer days of grazing for five heavily fertilized paddocks of Napier grass which were grazed rotationally during the 1938 to 1940 seasons and a portion of the 1937 season are given in Table 2. Three entire seasons of grazing for the five paddocks averaged 231 steer days grazing and 369 pounds of gain per acre. The average gain for the 3-year period was 1.60 pounds

TABLE 2.—*Animal days grazing, gain per acre, daily gains, and days grazing annually of heavily and lightly fertilized Napier grass when grazed rotationally.*

Field No.	Average per acre of heavily fertilized grass, 1938 thru 1940*		Average per acre of lightly fertilized grass, 1939 and 1940†	
	Steer days	Gain per acre‡	Heifer days	Gain per acre
1.....	253.0	401.0	128.5	180.5
2.....	252.3	423.0	155.5	220.0
3.....	241.7	376.7	168.0	237.0
4.....	233.7	368.3	168.0	239.0
5.....	176.3	274.3	153.0	219.5
Yearly av.....	231	369	155	219
Av. gain per day, lbs.....	1.60		1.41	
Av. grazing per season, days	165		175	

\*400 pounds of 5-7-5 fertilizer applied per acre in March each year. Additional nitrate of soda applied in 1938, 300 lbs.; 1939, 375 lbs.; and 210 lbs. in 1940.

†400 pounds of 5-7-5 fertilizer applied annually.

‡Gains per acre computed as follows: Animal days X daily gain for each season.

daily per steer. Best results were obtained in 1938 when steers gained 1.73 pounds daily and produced 430 pounds of beef per acre. The lowest gains resulted in 1940 when the production averaged 290 pounds of beef per acre. The lower production in 1940 was largely caused by decreased fertilizer applications, omission of cultivation resulting in subsequent plant competition, and cold injury of rhizomes which reduced the number of grass shoots.

The stand of grass was not as good in 1940 as in 1938. The retardation in stand was affected by differences in grazing. Since planting material was taken from mass selections of eyespot-resistant Napier grass, there were numerous plant variations in such characteristics as leafiness, pubescence, height and thickness of stems, and productivity. Observations showed that test animals had quite definite grazing preferences, resulting in overgrazing and subsequent reduction of root reserves and growth. This shows the desirability of using plants with uniform genotypes when conducting grazing tests with tall-growing grasses.

The growth and stand of grass was also retarded in some paddocks by excess moisture during certain seasons of the year. Improper drainage caused partial flooding of some paddocks in 1940.

In another experiment where Napier grass was fertilized lightly (400 pounds of a 5-7-5 fertilizer per acre annually), heifers gained 1.41 pounds daily. Two-year results show that 155 animal days of grazing were furnished per acre with 219 pounds of gain produced per acre (Table 2). A comparison of the beef produced on the heavily and lightly fertilized grass shows that the quantity of beef produced is largely dependent upon the amount of fertilizer applied.

In these two experiments Napier grass furnished good grazing, beginning in late April to early May until mid-October, or from 165 to 175 days annually (Table 2 and Fig. 3). Individual animal weights which were obtained each time the cattle were placed in a rested paddock showed gains in excess of 1 pound of beef per animal per day during the entire grazing season. This shows that the grass was maintained in a highly palatable and nutritious condition under the management practices used.

Considerable grazing was available after test animals were removed, but the forage was stemmy and coarse by the middle of October. After the test animals were removed, the grass was left ungrazed until late November to restore root reserves. This forage which accumulated after mid-October was grazed by the breeding herd and the data are not included in this paper.

Perhaps the most nearly similar data for comparing nutritive value of grasses for beef production, which were tested on different soils series and experiments, may be obtained by a comparison of daily gains of animals. Ritchey and Henley (11) report daily gains of steers which grazed on grasses commonly used in Florida as follows: 0.46 pound for carpet grass (*Axonopus compressus*); 0.66 pound for Bahia grass (*Paspalum notatum*); 0.61 pound for Bermuda grass (*Cynodon dactylon*); and 0.63 pound for a mixture of dallis (*Paspalum dilatatum*), Bermuda, carpet, and Bahia grasses. The animal daily gains produced from grazed Napier grass averaged 1.60 pounds for heavily

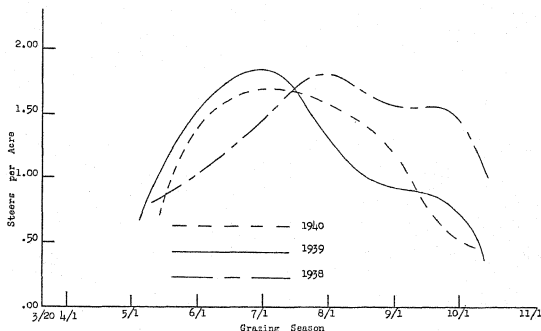


FIG. 3.—Daily change in grazing capacity (steers per acre) of heavily fertilized Napier grass during 1938, 1939, and 1940 seasons.

fertilized grass which was grazed by steers and 1.41 pounds for lightly fertilized Napier grass grazed by heifers.

#### SUMMARY AND CONCLUSIONS

Two areas of Napier grass were established on unproductive sandy soil types, fertilized differently, fenced into five paddocks, and grazed rotationally. Mass selections of disease-resistant types of Napier grass were used. Paddocks were stocked so that most grass blades were consumed in 5 to 8 days, thus allowing 20 or more days between grazings for the grass to recover.

The composition of Napier grass as managed for grazing was higher in dry matter and protein than grass managed for silage as reported by workers elsewhere.

The ungrazed residue of Napier grass (primarily stems) is inferior to consumed grass in protein, ash, calcium, phosphorus, fat, and fiber, but slightly higher in carbohydrates.

Highly fertilized grass produced an average of 231 animal days grazing, 369 pounds of beef per acre, and 1.60 pounds of gain daily for the 3-year period.

Lightly fertilized grass produced an average of 155 animal days grazing, 219 pounds of beef per acre, and 1.41 pounds gain daily for a 2-year period.

The desirability of using plants with uniform genotypes for grazing tests with tall growing grasses is suggested.

Napier grass as managed for grazing in these experiments is a very palatable and nutritious grass during the entire season, as indicated by daily animal gains in excess of 1 pound. It produced much higher daily gains than other grasses tested in Florida.

From the practical viewpoint Napier grass for grazing purposes appears especially desirable for use in fattening paddocks and for supplementary grazing.

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# INFLUENCE OF FERTILIZER AND TIME OF ITS APPLICATION ON GROWTH, YIELD, AND QUALITY OF PECANS<sup>1</sup>

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**A**PPPLICATION of fertilizer is an essential part of a well-planned program of orchard management for the production of pecans on the soils of the Southeast. Fertilizers, for the most part, are applied in early spring just before or at the time growth begins. Blackmon and Ruprecht (1)<sup>3</sup> obtained the highest yields on Bladen fine sandy loam soil when the fertilizers were applied in three or four different applications during the year. Later, Blackmon and Barnette (2) showed that summer applications of nitrogen fertilizers increases the yield of pecans over that produced with cover crops.

Skinner, *et al.* (4) found that the time of applying fertilizers in pecan orchards should vary according to the source of materials, climatic conditions, and general orchard practice. Their experimental evidence showed that plant food materials are most quickly assimilated during the period of rapid growth in early spring, but that increased yields resulted when additional light applications were made in May or June on deep sandy soils and in orchards where weeds or other plants were competing with the trees for nutrients. It is the purpose of this report to present results of an experiment designed to determine the effects of fertilizers applied at different times during the growing season on tree growth, yield, and quality of pecans.

## MATERIALS AND METHODS

The experiment is located on Greenville sandy loam soil in Lee County, Georgia.<sup>4</sup> The soil has a brownish-red surface and a red subsoil which is well-drained, but the subsoil contains considerable colloidal material and clay, thus making it quite compact in structure. These characteristics enable it to retain considerable moisture, a portion of which is not readily taken up by plants.

Trees of the Moore variety, planted 20 to the acre and 14 years before the beginning of this experiment, were selected for uniformity and divided into blocks of 10 trees each. These trees received an average of 20 pounds per tree of a 4.6-8.4-4 fertilizer annually during the five years preceding the experiment.<sup>5</sup> Be-

<sup>1</sup>The work reported in this paper was planned and conducted in the Division of Soil Fertility Investigations, Bureau of Plant Industry, U. S. Dept. of Agriculture, Oswald Schreiner, in charge, and the experiments were under the supervision of J. J. Skinner, formerly of this Division. Received for publication September 19, 1941.

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 187.

<sup>4</sup>W. M. Van Cise, Manager, Albany Peach and Pecan Company, contributed the use of the orchard and the labor for these experiments. His cooperation is appreciated. This experiment was inaugurated by E. D. Fowler, formerly of the Bureau of Plant Industry, now with the Soil Conservation Service, U. S. Dept. of Agriculture.

<sup>5</sup>The fertilizer constituents are given in the order of nitrogen, phosphoric acid, and potash.

ginning in the spring of 1935, the fertilizer was applied as follows: Block 1, all in February; block 2, one-half in February and one-half in April; block 3, one-half in February and one-half in June; block 4, one-half in February and one-half in August; and block 5, one-half in February and one-half in October. Two blocks were added in 1936, one with no fertilizer and one with one-sixth of the fertilizer applied in January, February, April, June, August, and October. All the fertilizer blocks received the same amount and kind of fertilizer.

At the beginning of the experiment a 6-8-4 fertilizer was applied at the rate of 60 pounds per tree, or 1,200 pounds per acre. In 1938, this was changed to a 4-8-4 at the rate of 50 pounds per tree, or 1,000 pounds per acre. From 1935 to 1937, 62.5% of the nitrogen in the fertilizer used was derived from nitrate of soda, 25% from sulfate of ammonia, and 12.5% from cottonseed meal. From 1938 to 1940, 15% of the nitrogen in the fertilizer was derived from nitrate of soda, 75% from sulfate of ammonia, and 10% from cottonseed meal. Superphosphate and muriate of potash were used to supply the phosphorus and potash, respectively. The fertilizers were made non-acid forming with dolomitic limestone.

Zinc sulfate at the rate of 5 pounds per tree was applied to all trees alike in 1937 and again in 1939 to keep the trees from developing rosette.

Winter green manure crops were grown each year. They consisted of a mixture of hairy vetch and rye from 1935 to 1937, rye alone in 1938, and a mixture of Austrian peas and rye in 1939. These crops were worked into the soil in the spring before serious competition for moisture developed between trees and green manure crops. The soil was cultivated during the summer months to keep down weeds and grass which would compete with the trees for moisture and nutrients.

The nut yield and trunk growth of the individual trees were measured annually. A portion of the nuts from the individual trees in each block were combined into a composite sample for physical and chemical analyses. From each composite sample, 150 nuts were taken and cracked to determine their conformity with the U. S. grade requirements; the remainder of the sample was sized to determine the proportions of the various sizes.

### RAINFALL

The rainfall data by weekly accumulations for the duration of the experiment, together with the normal rainfall, are shown in Fig. 1. Marked variations occurred in the distribution of the rainfall for the different seasons. During the growing season of 1935, the rainfall was below the average for the months of April, May, and June. From the first week in July to the second week in September, the rainfall was abundant, after which no effective rainfall was recorded.

During the growing season of 1936, the rainfall was above average in April. This was followed by an extended period of drought, lasting into the second week of July. Heavy rains occurred during the last of July and in early August, and more than an average amount was recorded for the rest of the season.

The growing season of 1937 had above average rainfall in April. During May and June, there were eight weeks of low rainfall. The rainfall for the rest of the season was near average and well distributed.

The rainfall for the growing season of 1938 was near average for the month of April; it was high in June and early July and low for August, September, and October.

The total rainfall for the growing season of 1939 was above average and was well distributed. It was not dry until October; and at such late period the dam-

aging effect was probably less than would have resulted from drought in any earlier period.

During the growing season of 1940, the rainfall was slightly above the average, and it was well distributed until the last of August. There was very little effective rainfall after that date.

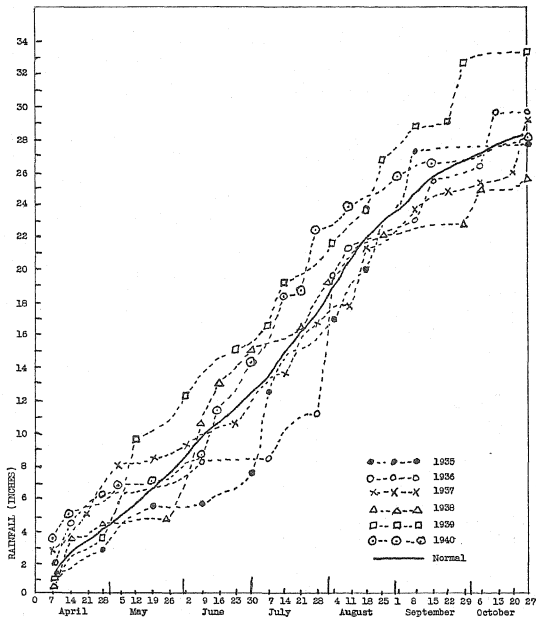


FIG. 1.—Cumulative rainfall curves for the six growing seasons, 1935-40, inclusive. Data from April 1 to October 27, inclusive, obtained at weekly intervals in the orchard where the experimental blocks were located. Normal curve based on the mean for 9 years, 1932-40, inclusive.

## EXPERIMENTAL RESULTS

### EFFECT ON GROWTH

The data on increase of the trunk cross-sectional area, together with the average cross-sectional area of the trees at the beginning of the experiment, are presented in Table 1. For the first two years the



trees receiving one-half of the fertilizer in February and one-half in June (block 3) made the greatest gain. In 1937 the trees receiving one-half of the fertilizer in February and one-half in August (block 4) made the greatest gain. For the last three years, the largest gains were made by the trees receiving all of the fertilizer in February (block 1). This latter group of trees were surpassed in average increase for the six years only by the trees in block 6 which received the six light applications during the season. The growth of the trees was more uniform during the last three years of the experiment on all blocks than it was during the first three years. This was probably due to the absence of a drought period in the early season of the last three years (Fig. 1). All fertilized trees made larger increases in cross-sectional area than the trees without fertilizer.

TABLE 1.—*Increase in trunk cross-sectional area of Moore pecan trees with fertilizer applied at different times.*

Block No.	Fertilizer treatment*	Average cross-sectional area, 1935†	Average increase in cross-sectional area per tree in sq. in. for							
			1935	1936	1937	1938	1939	1940	Total	Av.
1	All in February	51.8	9.2	15.5	9.6	16.4	15.9	19.8	86.4	14.4
2	½ in Feb.; ½ in Apr.	63.7	9.3	16.3	9.8	15.8	14.3	18.3	83.8	14.0
3	½ in Feb.; ½ in June	63.7	10.3	16.8	10.6	14.6	12.6	17.7	82.6	13.8
4	½ in Feb.; ½ in Aug.	57.1	8.9	12.5	11.8	16.3	14.4	19.7	83.6	13.9
5	½ in Feb.; ½ in Oct.	52.6	8.8	13.1	9.5	14.6	13.3	17.5	76.8	12.8
6	⅙ in Jan., Feb., Apr., June, Aug., and Oct.	—	—	16.4	10.5	15.8	13.9	19.7	76.3	15.3
7	No fertilizer	—	—	—	8.4	11.3	9.5	11.6	40.8	10.2

\*Fertilizer was 6-8-4 at the rate of 1,200 pounds per acre 1935 to 1937, inclusive, and 4-8-4 at the rate of 1,000 pounds per acre from 1938 to 1940, inclusive.

†Blocks 1 to 5 were all that were selected for the original experiment. The cross-sectional area of the trees in block 6 was 65.6 in the spring of 1936 and that for those in block 7 was 55.8 in the spring of 1937.

#### EFFECT ON YIELD OF NUTS

Data on the yield in pounds per tree for the individual trees are given in detail in Table 2. Probably the most outstanding feature of these data is the difference in the yields for alternate years and the progressive increase in yields for the trees in all the blocks during the "on-years". The size of the crop in 1940, an "off-year", is striking since the 1939 crop that preceded it was so large, and it stands out in marked contrast to the light crop produced in 1938 following the heavy crop of 1937.

The source of variance for the yield data and the standard error are shown in Table 3. The yields for the season of 1935 were not used in these calculations since no data were available for treatments 6 and 7 for that season.

All the fertilized trees produced significantly higher yields than did the unfertilized trees. The yields produced by the trees receiving fertilizer treatments do not show any significant differences between them.

#### EFFECT ON SIZE OF NUTS

Data on the percentage of the crop in the different sizes which have been averaged for the period of the experiment are given in Table 4. The nuts vary in size from 10/16 to 14/16 inch in diameter, with the bulk of the crop for all treatments falling in the 12/16 and 13/16 sizes. The unfertilized trees (block 7) produced fewer nuts in the two smaller sizes than did any block of fertilized trees. Block 3 which received one-half of the fertilizer in February and one-half in June shows the largest percentage of the crop falling in the two smaller sizes. Block 3 produced the highest average yield and block 7 produced the lowest average yield; therefore, it is indicated that there is an inverse relationship between the amount of the crop and the size of the nuts.

#### EFFECT ON QUALITY OF NUTS

In measuring the quality of the crop, the specific gravity and the percentage of well-filled nuts<sup>6</sup> were determined on the composite samples saved for this purpose. These data are tabulated in Tables 5 and 6. Because there was a change in both the amount and the composition of the fertilizer used during the period of 1935 to 1937 and the period of 1938 to 1940, the data are averaged for each of these two periods as well as for the entire period.<sup>7</sup> These two quality factors are so interrelated that it seems more convenient to discuss them together rather than separately.

Examination of the data in Tables 5 and 6 reveals that there is a direct correlation in most instances between specific gravity and the percentage of well-filled nuts. The variations in these two factors from season to season are quite marked, thus showing considerable difference in quality.

The differences between treatments are not consistent from season to season, but there is a tendency for the nuts of low specific gravity and the percentage of well-filled nuts to center around blocks 2 and 3 which received one-half of the fertilizer in February with the other half in April and June, respectively. This is particularly noticeable during the period from 1935 to 1937 and reached the peak in this respect in 1937. In that year, the nuts from block 3 were very poor with a specific gravity of 0.670 and only 14.7% of the nuts well filled. The nuts from block 2 were only slightly better, with a specific gravity of 0.685 and only 38.7% of the nuts well filled. The nuts from the other treatments varied in specific gravity from 0.720 to 0.735 and were from 56.7% to 67.3% well filled.

The average for the period of 1935 to 1937, inclusive, shows that the nuts from block 3 were decidedly inferior in quality to those from the other treatments and that those from block 2 were also inferior.

<sup>6</sup>By the term "well-filled" is meant those nuts which would meet the specifications set up for U. S. No. 1 grade by the U. S. Dept. of Agriculture.

<sup>7</sup>For these changes see section on "Materials and Methods".

TABLE 2.—*Effect of time of application of fertilizer on yield of pecan trees.*

Fertilizer treatment	Yield of nuts in pounds per tree for 10 trees								Sum	Mean
	1935*	1936	1937	1938	1939	1940				
1. All in Feb.	47.0 19.7 61.3 19.9 36.1 24.8 27.3 35.4 20.1 37.7	6.5 30.6 2.8 8.3 2.9 20.1 24.3 4.7 9.5 3.3	73.8 77.2 88.7 45.3 58.0 46.8 58.3 57.3 48.1 62.6	0.4 3.2 0.7 0.3 12.1 3.3 1.4 4.3 1.0 1.3	122.2 109.0 131.9 57.0 97.6 72.0 59.2 82.9 54.2 53.5	28.5 25.2 35.4 25.5 35.1 26.7 41.0 25.5 34.2 37.8			1,951.8	39.0†
2. ½ in Feb.; ½ in Apr.	28.9 0.5 31.5 40.0 47.5 2.0 39.0 27.7 31.9 31.1	21.4 4.6 4.1 23.1 1.6 19.0 12.4 17.7 32.2 16.0	65.0 43.1 36.2 75.0 90.8 74.2 71.3 56.0 86.9 68.4	3.5 0.1 2.0 0.4 0.5 0.3 0.2 0.0 2.6 0.6	85.2 43.4 58.3 79.9 111.7 74.7 95.8 48.4 101.0 64.2	17.3 38.8 22.1 58.5 20.0 57.6 15.0 60.5 46.8 41.2			1,969.6	39.4†
3. ½ in Feb.; ½ in June	66.5 42.5 50.5 49.9 41.5 40.6 56.4 40.5 54.4 28.3	16.0 13.5 7.2 40.2 27.1 5.8 19.8 10.7 19.8 24.8	86.0 61.8 55.8 78.4 85.8 53.8 74.1 62.5 72.3 40.6	0.4 2.4 0.0 3.8 2.2 0.3 0.7 0.0 0.2 4.0	87.2 74.0 72.2 108.4 86.8 55.9 79.0 66.3 83.3 54.3	42.4 29.0 27.9 29.6 42.6 35.5 46.4 44.8 56.3 23.5			2,015.4	40.3†
4. ½ in Feb.; ½ in Aug.	28.3 18.6 24.0 16.9 44.4 21.4 32.3 14.2 14.0 19.5	29.0 22.9 27.8 25.6 35.7 36.2 47.9 38.9 24.7 27.4	78.3 34.3 48.4 32.6 71.6 46.0 76.2 38.0 27.9 37.7	2.0 7.0 8.8 12.8 1.7 13.6 2.1 16.0 10.6 2.2	103.4 54.8 63.7 64.7 106.1 98.1 90.4 59.0 45.3 57.8	37.2 37.0 48.8 43.0 19.1 37.2 25.7 45.2 38.4 39.7			1,998.5	40.0†
5. ½ in Feb.; ½ in Oct.	45.8 22.5 23.0 44.0 37.3 36.3 45.8 23.0 31.8 22.0	3.9 19.2 5.3 37.0 22.9 11.8 22.8 29.9 15.1 29.7	73.4 37.5 75.6 84.7 63.4 57.7 81.1 32.4 37.2 35.5	0.3 8.3 0.4 1.3 4.9 0.0 0.9 11.7 14.0 31.1	101.3 60.2 75.9 85.7 74.5 62.1 94.9 54.2 51.2 69.3	33.3 31.8 42.5 47.7 30.9 31.1 26.3 33.0 44.1 43.7			1,942.7	38.9†

6. $\frac{1}{2}$ in Jan., Feb., Apr., June, Aug., and Oct.		—	—	—	2.0	0.7	61.3	34.8	3.1	13.7	88.8	66.2	11.4	6.2	1,822.8	36.5†
		—	—	—	4.7	1.4	73.5	92.0	6.3	0.7	110.9	122.6	38.8	30.5		
		—	—	—	17.1	1.1	47.3	78.8	4.1	1.5	54.7	108.7	17.5	13.9		
		—	—	—	1.9	2.9	44.1	54.3	1.0	20.0	83.6	103.7	2.8	13.8		
		—	—	—	0.2	9.2	62.7	72.9	2.1	8.6	83.9	111.3	30.0	19.5		
7. No fertilizer		—	—	—	14.0	10.8	5.1	11.5	21.5	31.7	55.5	44.1	6.5	49.9	1,357.9	27.2
		—	—	—	7.9	33.7	28.9	44.3	30.8	2.3	82.2	48.2	9.0	30.7		
		—	—	—	11.6	4.2	1.1	62.1	25.1	2.2	39.9	64.7	4.4	58.4		
		—	—	—	17.0	14.2	30.0	58.9	0.6	0.2	48.5	62.2	9.1	47.3		
		—	—	—	7.5	4.3	33.6	42.0	5.9	0.1	36.0	41.9	32.5	23.8		
Sum		—	—	—	1,130.1		3,962.8		383.4		5,320.0		2,262.4		13,058.7	
Mean		—	—	—	16.1		56.6		5.5		76.0		32.3			37.3

Correction factor, 487227.55.

\*The yields for 1935 were not included in the statistical treatment of the data.

†Significantly higher than no fertilizer.

TABLE 3.—*Source of variance.*

Variance due to	DF	Sum of squares	Variance
Total.....	349	339,459.60	
Between years.....	4	6,477.69	1,619.42
Between treatments.....	6	234,897.58	39,149.60
Treatment X years.....	24	65,293.50	2,720.56
Error.....	315	32,790.83	104.10

Standard error of sum for a treatment for 1 year =  $\sqrt{104.1 \times 10} = \sqrt{1041.00} = 32.2$ .

Standard error of a mean for a treatment for the 5-year period =  $\sqrt{104.10} = \sqrt{2.08} = 1.44$ .

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The variations in specific gravity and percentage of well-filled nuts are not as marked for the period of 1938 to 1940 as they were for the first period. The averages for this period show that the nuts from block 3 continued to have the lowest specific gravity and that those from block 2 were second in this respect. The percentage of well-filled nuts does not correlate perfectly with the specific gravity in these average figures since block 5, receiving one-half of the fertilizer in February and one-half in October, was lower in the percentage of well-filled nuts but higher in the specific gravity of the nuts than were block 2 and 3. The nuts from all treatments during this last 3-year period were decidedly superior in quality to those produced during the first 3-year period.

Considering all the data, it is indicated that the quality of the nuts has been lower in crops produced with fertilizers applied one-half in February and the other half in either April or June than from fertilizers applied at other times. This is of particular interest since April to June is the period of active vegetative growth in the pecan tree. The nuts produced on unfertilized trees have been slightly superior in quality to those produced on any of the fertilized trees.

TABLE 4.—*Percentage of pecans in the different sizes from trees fertilized at different times, 6-year average.*

Block No.	Fertilizer treatment*	Percentage of nuts in size				
		10/16 in. dia.	11/16 in. dia.	12/16 in. dia.	13/16 in. dia.	14/16 in. dia.
1	All in Feb.	0.8	15.1	39.5	34.5	10.1
2	½ in Feb.; ½ in Apr.	0.6	10.5	43.8	34.5	10.6
3	½ in Feb.; ½ in June	1.3	18.1	41.9	30.6	8.1
4	½ in Feb.; ½ in Aug.	0.7	11.2	45.7	34.2	8.2
5	½ in Feb.; ½ in Oct.	0.7	12.3	45.0	33.7	8.3
6†	½ in Jan., Feb., Apr., June, Aug., and Oct.	0.5	10.8	48.8	32.4	7.5
7†	No fertilizer	0.2	5.9	49.1	34.5	10.3

\*Fertilizer was 6-8-4 at the rate of 1,200 pounds per acre from 1935 to 1937, inclusive, and 4-8-4 at rate of 1,000 pounds per acre from 1938 to 1940, inclusive.

†Average for 5 years.

TABLE 5.—*The specific gravity of pecans from trees fertilized at different times.*

Block No.	Fertilizer treatment*	Specific gravity								
		1935	1936	1937	Av., 1935-37	1938	1939	1940	Av., 1938-40	Av., 1935-40
1	All in Feb.	0.740	0.667	0.720	0.709	0.744	0.756	0.731	0.744	0.726
2	$\frac{1}{2}$ in Feb.; $\frac{1}{2}$ in Apr.	0.755	0.662	0.685	0.701	0.736	0.758	0.695	0.730	0.715
3	$\frac{1}{2}$ in Feb.; $\frac{1}{2}$ in June	0.730	0.675	0.670	0.692	0.720	0.765	0.703	0.729	0.711
4	$\frac{1}{2}$ in Feb.; $\frac{1}{2}$ in Aug.	0.765	0.673	0.730	0.723	0.734	0.768	0.744	0.749	0.736
5	$\frac{1}{2}$ in Feb.; $\frac{1}{2}$ in Oct.	0.745	0.675	0.720	0.713	0.717	0.771	0.727	0.738	0.726
6	$\frac{1}{6}$ in Jan., Feb., Apr., June, Aug., and Oct.	—	0.660	0.735	0.698	0.719	0.753	0.778	0.750	0.729
7	No fertilizer	—	0.679	0.730	0.705	0.734	0.797	0.767	0.766	0.741

\*Fertilizer was 6-8-4 at the rate of 1,200 pounds per acre from 1935 to 1937, inclusive, and 4-8-4 at rate of 1,000 pounds per acre from 1938 to 1940, inclusive.

TABLE 6.—*The percentage of well-filled pecans from trees fertilized at different times.\**

Block No.	Fertilizer treatment†	Percentage of well-filled nuts								
		1935	1936	1937	Av., 1935-37	1938	1939	1940	Av., 1938-40	Av., 1935-40
1	All in Feb.	80.7	36.7	64.7	60.7	82.0	84.0	55.3	73.8	67.2
2	½ in Feb.; ½ in Apr.	70.7	39.3	38.7	49.5	76.0	80.0	33.3	63.1	56.3
3	½ in Feb.; ½ in June	60.3	44.4	14.7	39.8	72.0	89.3	38.0	66.4	53.1
4	¼ in Feb.; ¼ in Aug.	80.7	38.9	58.0	59.2	72.6	88.0	60.0	73.5	66.4
5	½ in Feb.; ½ in Oct.	69.7	39.6	61.3	52.9	51.3	88.7	36.7	58.9	57.9
6	⅙ in Jan., Feb., Apr., June, Aug., and Oct.	—	36.0	67.3	51.9	70.7	90.7	92.0	84.5	71.3
7	No fertilizer	—	39.1	56.7	47.9	72.0	99.3	92.0	87.8	71.8

\*By well-filled nuts is meant those that would meet the specifications set up for U. S. No. 1 grade by the U. S. Dept. of Agriculture.

†Fertilizer was 6-8-4 at the rate of 1,200 pounds per acre from 1935 to 1937, inclusive, and 4-8-4 at the rate of 1,000 pounds per acre from 1938 to 1940, inclusive.

## OIL AND PROTEIN

Data on the oil and protein content<sup>8</sup> of the nut kernels produced during the "on-years" of 1937 and 1939 are given in Table 7. The variations in these two factors are quite marked. The oil content for the 1937 crop is considerably lower and the protein content considerably higher than for the 1939 crop.

TABLE 7.—*The oil and protein content of the pecan crops of 1937 and 1939.\**

Block No.	Fertilizer treatment†	1937		1939	
		Oil, %	Protein, %	Oil, %	Protein, %
1	All in Feb.	66.6	12.72	71.7	11.17
2	½ in Feb.; ½ in Apr.	65.7	12.29	72.0	10.76
3	½ in Feb.; ½ in June	65.7	13.57	73.4	10.70
4	½ in Feb.; ½ in Aug.	68.9	12.98	72.9	10.82
5	½ in Feb.; ½ in Oct.	69.2	12.40	73.1	10.53
6	½ in Jan., Feb., Apr., June, Aug., and Oct.	66.8	12.77	72.3	10.56
7	No fertilizer	67.9	12.24	75.1	9.31

\*Analyses for oil and protein were made on a combined sample of the U. S. No. 1 and U. S. No. 2 kernels.

†The fertilizer was 6-8-4 at the rate of 1,200 pounds per acre from 1935 to 1937, inclusive, and 4-8-4 at the rate of 1,000 pounds per acre from 1938 to 1940, inclusive.

There does not seem to be any consistent relationship between the time the fertilizers were applied and the content of oil or of protein of the pecan kernels produced. In view of the discussion already presented, it is of some importance to point out that in oil content the nuts from block 3 in 1937, which have been shown to be poorer in quality than any other sample, do not differ greatly from the other samples of that year, yet the protein content is considerably higher. These factors will be discussed in more detail later.

## DISCUSSION

There are numerous environmental factors which determine the quality of any crop. The data presented herein are the result of an effort to evaluate one of these factors for pecans. Doubtless other environmental factors not under control have had a marked effect on the outcome of these investigations. Investigators have reported on the influence of fertilizers, soil moisture, leaf area, and size of crop in their relation to quality of the pecan. Finch and Van Horn (3) reviewed the literature on these subjects and point out that there is much confusion and chaos in our present knowledge on the subject of nut filling. In their studies they found that pecan filling and maturity were influenced by the vegetative condition of the tree, that trees which are moderately vegetative store more starch in the shoots in early summer, and that starch storage during the period preceding filling is associated with better quality nuts.

\*These analyses were made on combined samples of the U. S. No. 1 and U. S. No. 2 kernels.

It is the belief of the writers that the poor quality nuts produced by trees which received fertilizer during April and June might be the result of stimulated vegetative activity at this time, thus preventing the accumulation of starch needed for the filling process at a later period.

The quality of the crops produced during the period of 1935 to 1937 when 1,200 pounds per acre of 6-8-4 fertilizer were applied as compared with the period of 1938 to 1940 when 1,000 pounds per acre of 4-8-4 fertilizer were applied, show considerable advantage for the latter period (Table 5 and 6).<sup>9</sup> It was during the latter period preceding the crop of 1939 that a rye green manure crop was substituted for the legumes.<sup>10</sup> Thus, the nitrogen available to the trees during the first three years of the experiment was considerably higher than during the last three. The best quality nuts produced during the period under study were associated with a moderate nitrogen supply to the trees.

The oil and the protein content of the crops of 1937 and 1939 (Table 7) are of interest when considered from this standpoint. The 1937 crop which was produced following a 3-year use of a high-nitrogen fertilizer along with legume green manure crops was high in protein and low in oil content, whereas the 1939 crop which was produced following a 2-year use of a moderately low nitrogen fertilizer and a rye green manure crop immediately preceding it was comparatively low in protein and high in oil content.

The increase in the amount of sulfate of ammonia in the fertilizer during the last 3 years, with the necessary increase in the amount of dolomitic limestone to make it non-acid forming, resulted in a larger supply of calcium, magnesium, and sulfur to the trees during the last half of the test period than for the first half. The importance of these three elements in the nutrition of certain plants is well known, but their importance in the nutrition of the pecan has not been established. The fact that the quality of the crops was much better during the last half of the test period indicates that the increased amount of these elements in the fertilizer may have contributed to this. Magnesium is known to be higher in oil-bearing seeds than in starch-bearing seeds and this may have been the important element affecting quality.

The usual concept is that August and September are critical months so far as moisture is concerned in its relation to nut filling. There is very little in these data which would indicate that August and September are more important in this respect than are the months of May, June, and July. A critical examination of the rainfall data (Fig. 1) in its relation to pecan quality (Tables 5 and 6) indicates that poorer filled nuts are produced when grown during a season with a long period of low rainfall during May, June, and July followed by an abundance of moisture thereafter than are produced during a

<sup>9</sup>These changes in the fertilizer resulted in a 44% reduction in the commercial nitrogen used.

<sup>10</sup>Yield of legumes and analyses made on some of the crops indicate that nitrogen equivalent to 400 or 500 pounds of a fertilizer containing 16% to 20% nitrogen was added to the soil in the legume crop.



season with an abundance of moisture in May, June, and July followed by a long period of low rainfall in August, September, and October.

It is conceivable that a period of low rainfall during the early months of the growing season might delay vegetative development to such a degree that an abundance of moisture which followed might cause the trees to continue vegetative activity too late in the season for the trees to accumulate the necessary reserves of elaborated foods to effect the proper filling of the nuts.

From the foregoing discussions, the evidence indicates that fertilizers and rainfall distribution have exerted an influence on pecan quality, and that when either, or both, are conducive to late season vegetativeness in the trees, they may cause a lowering in quality. These factors are of sufficient practical importance that further investigations are needed to evaluate their significance.

### SUMMARY

The tree growth and nut yield and quality of the Moore pecan grown on Greenville sandy loam soil with fertilizer applied at different times are reported. Fertilizers were applied as follows: All in February; one-half in February and one-half in April; one-half in February and one-half in June; one-half in February and one-half in August; one-half in February and one-half in October; and  $1/6$  in January, February, April, June, August, and October. A plot with no fertilizer was included as a check. All treatments and the check included the same winter green manure crops and cultivation.

Tree growth was increased by all fertilizer treatments, the largest average increase being from fertilizer applied in six light applications.

Nut yields were significantly increased by all fertilizer treatments when compared to unfertilized trees. Time of fertilizer application had no significant influence on yield.

There was an inverse correlation between the yield and the size of the nuts.

The quality of the nuts, as measured in terms of specific gravity and percentage of well-filled nuts, was influenced by the time of the fertilizer application. The nuts of poorest quality were produced on the trees receiving one-half of the fertilizer in February and one-half in June; and those of best quality were produced on unfertilized trees.

The oil and the protein content for the two "on-year" crops of 1937 and 1939 varied widely for the two seasons. There was less variation in these constituents due to fertilizer treatments, and no definite relationship with such treatments was evident.

Fertilizer applied in split applications at different times during the season has not given significantly higher yields than when it was applied in a single application in February; and the quality of the nuts produced from this latter method compared favorably with the best quality produced with the former. Therefore, the one single application made in February might well be adopted on the basis of economy.

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## RELATION OF EXCHANGEABLE POTASSIUM IN ALABAMA SOILS TO NEEDS OF THE COTTON CROP<sup>1</sup>

N. J. VOLK<sup>2</sup>

PLANTS growing in a soil obtain potassium from the soil solution, exchange material, other secondary as well as primary minerals, and organic matter. A great many workers (1, 2, 3, 4, 5, 6, 7)<sup>3</sup> have investigated or developed methods of extracting and determining the amount of potassium in a soil that may be available to plants. From the data obtained, attempts have been made to show a relationship between the potassium thus extracted from the soil and the need for potassium fertilization. For a number of crops and soils there appears to be a close relationship between yield and exchangeable potassium. Since the equivalent of about 45,000 tons of muriate of potash is used annually as a fertilizer in Alabama and since about 70% of this is used for the growing of cotton, a study was undertaken to determine whether any relation exists between exchangeable potassium in Alabama soils and the response of cotton to potash fertilization.

During the years 1937 to 1940, inclusive, 113 cooperative tests were conducted by the Alabama Agricultural Experiment Station<sup>4</sup> for the purpose of studying rates of potash fertilization for cotton in certain cropping systems. These tests covered a wide variety of soils and revealed that 94% of the soils responded significantly to the first increment of 25 pounds of potash per acre. The average increase in the yield of seed cotton for this increment was 195 pounds per acre. On the other hand, only 55% of the soils responded significantly to a second increment of potash, and the average increase in the yield of seed cotton from this was 62 pounds per acre. In another investigation<sup>5</sup> involving 283 cooperative tests, it was found that 41% of the soils responded significantly to a second increment of potash (25 pounds of K<sub>2</sub>O per acre). No information was obtained regarding the response to the first increment of potash since all plots received at least 25 pounds of potash per acre.

### RELATION OF EXCHANGEABLE POTASH TO YIELD OF SEED COTTON

The data used in the present study were obtained from the sub-stations and experiment fields of Alabama. These fields were selected because of the uniformity of the physical composition of the soil, level topography, and the importance of the soil type as an agri-

<sup>1</sup>Contribution from the Department of Agronomy and Soils, Alabama Agricultural Experiment Station, Auburn, Ala. Published with the approval of the Director. Received for publication October 20, 1941.

<sup>2</sup>Soil Chemist.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 198.

<sup>4</sup>Cooperative tests conducted by Dr. J. W. Tidmore, Assistant Director, Alabama Agricultural Experiment Station.

<sup>5</sup>Cooperative tests conducted by Mr. J. T. Williamson, Associate Agronomist, Alabama Agricultural Experiment Station.

cultural soil in the state. In all of the plots involved, the need for nitrogen and phosphorus was eliminated by adequate additions.

The relation between the exchangeable potash in the soil and the yield of seed cotton obtained is shown in Figs. 1 and 2. These scatter diagrams show that for soils containing less than about 200 pounds of exchangeable potash per acre, a general relationship exists between the amounts of exchangeable potash in the soils and the yields of seed cotton obtained. It will be noted that in a few cases there appears to occur a depression in the yields where the potash contents are high. This was probably caused by greatly increased boll weevil injury of the late-maturing cotton which is often produced when the supply of available potash is high. It has been demonstrated that high potash will cause late maturity which may result in enhanced boll weevil injury. For the soils having more than 200 pounds per acre of exchangeable potash, the relationship between the amounts of exchangeable potash and the yields of seed cotton is poor or entirely lacking as in the case of the Prattville experiment field. However, cotton on these soils responds to light applications of potash. The data presented in Table 1 are further proof that increased yields resulting from applied potash are not limited to soils containing less than 200 pounds of exchangeable potash per acre.

TABLE 1.—*The amount of exchangeable potash in the soil and the increase in the yield of seed cotton resulting from the application of 25 pounds of potash, 1930 to 1938, inclusive.\**

Soil type†	Pounds per acre of exchangeable potash	Pounds per acre increase in the yield of seed cotton resulting from the application of 25 pounds of potash	Soil type	Pounds per acre of exchangeable potash	Pounds per acre increase in the yield of seed cotton resulting from the application of 25 pounds of potash
Norfolk SL	46	443	Norfolk FSL	120	81
Hartsells VFSL	63	278	Kalmia VFSL	124	261
Hartsells VFSL	65	134	Greenville FSL	161	280
Norfolk SL	68	326	Greenville clay	198	156
Hartsells VFSL	69	181	Greenville clay	251	83
Hartsells VFSL	75	337	Decatur clay	274	53
Hartsells VFSL	77	185	Decatur clay	304	100
Norfolk FSL	77	299	Greenville clay	306	79
Norfolk FSL	79	183	Decatur clay	306	65
Norfolk FSL	85	290	Decatur clay	312	364
Hartsells VFSL	85	175	Decatur clay	323	292
Norfolk FSL	93	293	Decatur clay	342	75
Greenville FSL	94	153	Decatur clay	342	164
Norfolk FSL	98	170	Decatur clay	351	41
Norfolk FSL	107	268	Decatur clay	355	63
Kalmia VFSL	108	98	Decatur clay	391	24
Hartsells VFSL	112	401	Decatur clay	461	27

\*Results obtained from substations and experiment fields.

†SL = Sandy loam; VFSL = Very fine sandy loam; FSL = Fine sandy loam.

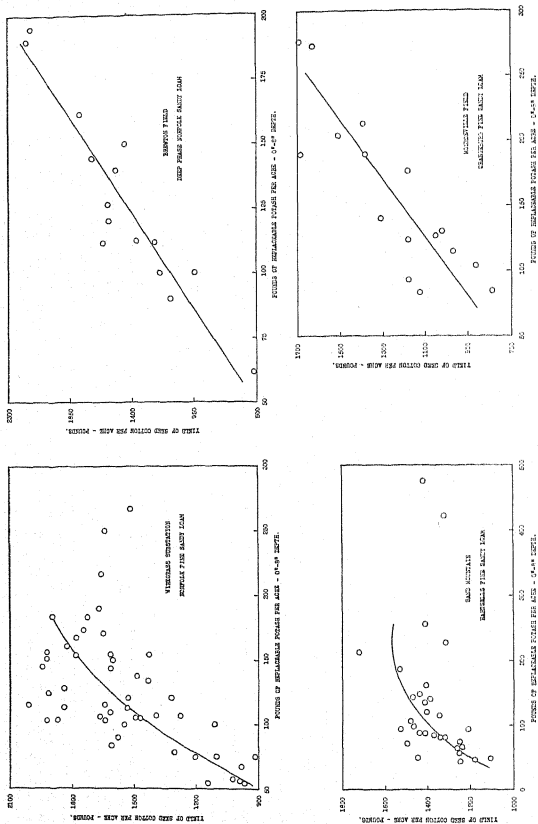


FIG. 1.—The relation between the yield of seed cotton and the amount of exchangeable potash in the soils of some of the substations and experiment field plots.

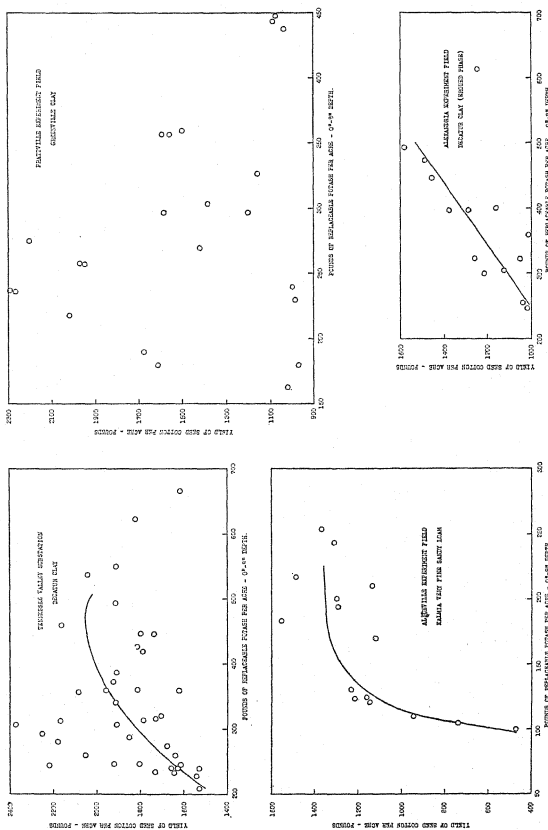


Fig. 2.—The relation between the yield of seed cotton and the amount of exchangeable potash in the soils of some of the substitution and experiment field plots.

It is apparent from the data at hand that about 95% of all soils in Alabama which are suited to the production of cotton respond significantly to the application of the first increment (25 pounds per acre  $K_2O$ ) of potash, but the magnitude of the response varies tremendously and is dependent on various soil, climatic, and cultural conditions. Little or no need for a laboratory method for determining the need of this first increment could therefore exist. On the other hand, only about 40% to 50% of the soils respond significantly to the application of a second increment of potash. A chemical method for determining available potassium in soils would be valuable if the results of such analyses would aid in determining with a fair degree of accuracy those soils which would respond to the application of a second increment of potash.

#### RELATION OF EXCHANGEABLE POTASH TO YIELD OF SEED COTTON RESULTING FROM A SECOND INCREMENT OF POTASH

For this study, the yields from 283 cooperative tests representing most of the important soil types in Alabama were used (Tables 3 and 4). Each of the cooperative tests consisted of several plots,  $1/30$  acre in size, all of which received an adequate supply of nitrogen and phosphorus but different amounts of potash. Representative samples of soil were obtained before any fertilizer was applied by taking 10 to 20 borings from each plot to a depth of 7 to 8 inches. The samples were air dried, thoroughly mixed, put through a 30-mesh sieve, and analyzed for exchangeable potash by means of a method described elsewhere (8). Yields of seed cotton were obtained from 1937 to 1939, inclusive; only the first year's yields from any given set of plots were used in the present consideration so as not to involve the residual effect of the potash applied to the treated plots. All tests (about 15% of the total) which were unsatisfactory because of low yields or nonuniformity of the check plots were discarded. This procedure was adopted in order to eliminate as far as possible the limiting factors not foreseen at the start of the experiment, such as a deficiency of minor nutrient elements, or inherent soil differences not readily detectable.

A summary of the results obtained is given in Table 2. It is apparent that when the soils are arranged according to their content of exchangeable potash without regard for soil series or texture, no sharp division exists between those which respond to the second increment of potash and those which do not, but there is a trend toward greater response and greater frequency of response when the exchangeable potash is low. Thus, the content of exchangeable potash cannot be used as the sole criterion for classifying soils as regards their need for a second increment of potash.

In an attempt to reveal any relationship that might exist between the amounts of exchangeable potash in Alabama soils and a significant increase in the yield of seed cotton obtained from the second increment of potash, the data have been grouped in various ways.

TABLE 2.—*The distribution of 283 cooperative field tests grouped according to the exchangeable potash content of the soils and the response in seed cotton to an application of more than 25 pounds per acre of potash.\**

Pounds per acre of exchangeable potash in the soil groups	Number of field tests conducted on each group of soils	Percentage of the tests that responded significantly to an application of more than 25 pounds per acre of potash	Average increase in pounds of seed cotton per acre for the tests showing a significant response to an application of more than 25 pounds per acre of potash
18-25	6	100	106
26-50	39	62	99
51-75	44	50	81
76-100	21	48	87
101-125	56	34	104
126-150	16	18	37
151-200	47	38	80
201-250	25	32	53
251+	29	28	58
Averages . . . . .	—	41	84

\*Practically all Alabama soils show a profitable response to 25 pounds of potash and it would be valuable to be able to determine by laboratory methods the soils that would respond profitably to more than 25 pounds of potash. More than 25 pounds of seed cotton per 12½ pounds of applied potash is a significant response.

In Table 3 they are grouped according to soil series and the exchangeable potash content, but without regard to soil texture. From the averages presented for each soil series group, it is evident that more than half of the soils in the Norfolk and Clarksville groups and less than half of the soils of the Hartsells, Cecil, Decatur, and Orangeburg groups responded to the second increment of potash. It is apparent that some significance can be attached to the exchangeable potash content of the Norfolk group of soils and the resultant response to the second increment of potash. For the other five soil series groups apparently no significance can be attached to the content of exchangeable potash. All of the soils of the Norfolk group containing less than 26 pounds of exchangeable potash per acre responded to the second increment of potash, while about 60% of those containing between 26 and 100 pounds responded and only about 25% of those containing over 100 pounds responded.

A grouping of the soils according to soil texture and the exchangeable potash content, but disregarding soil series, is presented in Table 4. As would be expected, the percentage of soils responding to the second increment of potash increased as the texture became coarser. For the soils studied, 49% of the sands and only 29% of the clays responded significantly.

In all cases except for the clay group there is a definite trend toward a decrease in the percentage of soils responding to the second increment of potash with an increase in the amount of exchangeable potash. On the other hand, the percentage of soils not responding in each grouping is so large that satisfactory predictions regarding the probable response are impossible if only these factors are considered.



TABLE 3.—*The relation of soil series and exchangeable potash to the response of cotton resulting from the application of a second increment (25 pounds of  $K_2O$  per acre) of potash.*

Pounds per acre of exchangeable potash in the soil	Number of field tests conducted	Response to application of second increment of potash	
		Percentage of tests that responded significantly	Increased yield, pounds of seed cotton per acre
Norfolk Group*			
18-25	6	100	106
26-50	32	66	100
51-75	29	52	74
76-100	8	75	62
101-125	13	31	85
126+	5	20	40
Averages.....	—	57	76
Clarksville Group			
68-100	7	57	98
101-125	7	86	101
126-175	9	22	60
176+	6	67	94
Averages.....	—	55	94
Hartsells Group			
54-100	9	33	170
101-125	13	46	158
126+	6	17	110
Averages.....	—	36	157
Cecil Group			
32-75	12	25	44
76-100	4	50	105
101-150	6	50	37
151+	6	17	30
Averages.....	—	32	53
Decatur Group			
108-150	9	33	143
151-200	16	25	92
201-250	19	37	60
251-300	10	30	40
301+	13	23	78
Averages.....	—	30	78
Orangeburg Group			
83-100	3	33	100
101-125	14	0	0
126-175	8	50	57
176+	13	51	70
Averages.....	—	24	67
Av. for all groups	—	41	84

\*Norfolk group contains Norfolk, Ruston, and Kalmia; Clarksville group, Clarksville, Colbert, and Susquehanna; Hartsells group, Hartsells and Hanceville; Cecil group, Cecil, Durham, Louisa, and Appling; Decatur group, Decatur, Dewey, and Holston; Orangeburg group, Orangeburg, Red Bay, Greenville, Cahaba, and Amite.

TABLE 4.—*The relation of soil texture and exchangeable potash to the response of cotton resulting from the application of a second increment (25 pounds of  $K_2O$  per acre) of potash.*

Pounds per acre of exchangeable potash in the soil	Number of field tests conducted	Response to application of second incre- ment of potash	
		Percentage of tests that responded significantly	Increased yield, pounds of seed cotton per acre
Sand Group*			
18-25	6	100	106
26-50	31	58	95
51-75	22	45	68
76-125	16	31	39
126+	6	17	30
Averages.....	—	49	82
Sandy Loam Group			
29-50	7	71	101
51-75	17	59	74
76-100	12	42	82
101-125	21	33	87
126-175	12	33	80
176+	5	20	40
Averages.....	—	44	82
Loam Group			
50-75	5	60	171
76-100	4	50	130
101-150	25	40	109
151-200	20	35	77
201+	12	42	46
Averages.....	—	42	94
Clay Group			
68-150	9	33	107
151-200	17	29	92
201-250	19	37	60
251+	17	18	78
Averages.....	—	29	80
Av. for all groups	—	41	84

\*Sand group contains sands, loamy sands, and sandy loam; sandy loam group, fine sandy loam and very fine sandy loams; loam group, loams, sandy clay loams, clay loams, and silt loams; clay group, sandy clay, silty clay, silty clay loam, and clay.

## DISCUSSION

The differences in cotton yields so frequently obtained from plots on a given experimental field which have been fertilized uniformly with nitrogen, phosphorus, and potash are very likely due to several factors, the most important of which are (a) variations in previous

cultural practices, (b) variations in the organic matter content of the soil, (c) variations in the compactness of the immediate subsoil layer, (d) the kind of crop grown the previous year or two, (e) available nutrients in the subsoil, (f) variations in the amount of native available plant food in the soil, and (g) the planting date.

Previous cultural practices influence the yield of cotton. Peanuts harvested (not hogged) ahead of cotton for several years often injure the soil for the cotton that follows for a number of years (9). Many Alabama soils gradually increase in productivity where legumes are grown to supply the nitrogen. A simple rotation of cotton and corn seems to have some beneficial effect on the yield of cotton as compared to yields obtained from continuous cotton.

Some of the soils of Alabama are so devoid of organic matter that they have become practically sterile. Studies associated with oxidation and reduction showed that readily decomposable organic matter applied to some soils in Alabama will remain in an unaltered state for several days to a week, while in other soils decomposition sets in almost at once. Apparently, the number of microorganisms in some soils is limited. In such cases, commercial fertilizers might be less effective in producing maximum returns.

It is a common occurrence for a field to exhibit very uneven growths of cotton, corn, sorghum, or other crops. Just why corn will be 7 feet high in one place and 15 feet away it will be 2 feet high is not clearly understood. Certainly it is not the lack of the ordinary plant food elements, N, P, and K, because in most cases the entire field received an adequate and uniform application of these elements. Evidence at hand indicates that some soils are becoming exhausted of so-called minor elements. The variation in the compactness of the immediate subsoil layer causes differences in the amount of moisture that is available to the plants. Variations in organic matter content and previous cultural practices also have their influence. Experiments in progress at this Station (9) reveal that plants can remove potash in amounts considerably in excess of that extracted at any one time by ammonium acetate, and that soils differ greatly in their ability to maintain a supply of exchangeable potash.

Another factor of considerable importance is the boll weevil. During years when the boll weevil practically ruins a late cotton crop, potash additions, especially if excessive, by causing late maturity tend greatly to increase the resulting damage. Plots receiving 96 pounds of potash per acre consistently produce less cotton in certain regions than do those receiving 48 pounds, and quite often the plots receiving 48 pounds produce less cotton than those receiving 24 pounds. Planting cotton too late is the cause of a great many failures resulting from boll weevil injury.

It may be that crops other than cotton grown in Alabama would show a better relation between the amounts of native exchangeable potash in the soil and the need for potash; but for cotton, no well-defined relation appears to exist which would enable the investigator to predict with reasonable assurance when a soil would respond profitably to a second increment of potash. (An increment of potash is 25 pounds of  $K_2O$  per acre.) It should not be concluded from the

results of this investigation that two soils identical in every respect except content of exchangeable potash would not produce cotton in conformity to their contents of exchangeable potash, providing other plant food elements were present in sufficient quantities. It is concluded from these results, however, that differences in response of cotton to like amounts of potash in different soils are the result of differences in soil characteristics measurable or not measurable, recognizable or not recognizable, which have a profound influence on the final yield obtained.

### SUMMARY AND CONCLUSIONS

In an attempt to reveal any relationship that might exist between the amount of exchangeable potash in Alabama soils and the increased yield of seed cotton resulting from the application of different amounts of potash, a study was made of the results obtained from 599 substation, experimental field, and cooperative fertilizer tests distributed over the entire state. The effect of soil series and texture on the above relationship was also investigated. The results may be summarized as follows:

1. About 95% of all soils studied responded significantly to the first increment (25 pounds per acre of  $K_2O$ ) of applied potash and about 40% to 50% responded significantly to the second increment of applied potash.
2. For soils of the same type containing less than about 200 pounds of exchangeable potash per acre, there is a general relationship between the total yield of seed cotton and the total amount of exchangeable potash contained in the soil. Soils, however, which contained over 200 pounds of exchangeable potash per acre frequently responded to an application of potash.
3. A laboratory method to aid in determining which soils do not respond to the application of the first increment of potash would have little application in the case of cotton in Alabama since only about 5% of all the soils of the state are involved. On the other hand, any method which would indicate with a fair degree of accuracy those soils which respond significantly to the application of a second increment of potash would be extremely valuable.
4. The results of this investigation reveal that predictions of the need of the cotton plant for a second increment of potash, based on a knowledge of the texture, series, and exchangeable potash content of the soils, will be unreliable in about 35% of the cases. This is due in many cases to the fact that the additional potash causes greater growth, later maturity, and, as a consequence, more serious boll weevil injury.
5. It is believed that differences in response to like amounts of potash are caused by differences in soil characteristics which may greatly influence the yield of cotton. If possible, these should be taken into consideration in making fertilizer recommendations on the basis of soil analysis.

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## NOTES

### A RAPID METHOD FOR DETERMINING HYDROCYANIC ACID CONTENT OF SINGLE PLANTS OF SUDAN GRASS

A RAPID method for determining the amount of hydrocyanic acid in green plant material based on the picric acid test was proposed by Pethybridge<sup>1</sup> in 1919. The use of chloroform in the test was suggested by Mirande,<sup>2</sup> while Nowosad and MacVicar<sup>3</sup> later substituted toluene. The procedure used at the Wisconsin Agricultural Experiment Station for testing single plants of sudan grass is a modification of the method devised by Nowosad and MacVicar.

The method consists of placing 0.15 gram of green plant material, cut into short pieces with a scissors or macerated, in a test tube, adding 3 or 4 drops of chloroform, and suspending a strip of moist filter paper saturated with sodium picrate solution above the mixture. The saturated filter paper is held in place with a cork stopper which also serves to seal the test tube. The tube with contents is incubated at room temperature (20° C) for 12 to 24 hours. The sodium picrate present in the filter paper is reduced to a reddish compound in proportion to the amount of hydrocyanic acid evolved. The color produced is dissolved by placing the paper in a clean test tube containing 10 cc of distilled water after which the color of the water is matched with color standards.

The test is sufficiently quantitative to serve as a basis for the selection of plants low in hydrocyanic acid. The results may be expressed in relative terms such as "high", "medium", or "low" or in approximate p.p.m. based on the percentage dry matter in the sample.

As test material, actively growing, vigorous tillers from 5 to 7 inches in length can be used regardless of the height of growth of the remaining portions of the plant. The samples for analysis for hydrocyanic acid are taken from that portion of the tiller immediately below the uppermost leaf collar.

### REAGENTS AND STANDARDS

*Alkaline picrate solution.*—Dissolve 25 grams of  $\text{Na}_2\text{CO}_3$  and 5 grams of picric acid (C.P.) in 1,000 cc of distilled water.

*Chloroform.*—U.S.P. grade.

*Color standards.*—Dissolve 0.241 gram KCN in 1,000 cc of water. This gives a stock solution containing 0.1 mg HCN per cc. Place 5 cc of the alkaline picrate solution and 5 cc of the KCN solution in a test tube. Heat for 5 minutes in boiling water. Add the following

<sup>1</sup>PETHYBRIDGE, G. H. Is it possible to distinguish the seed of wild white clover by chemical means during the germination test? Econ. Proc. Roy. Dublin Soc., 2:248-258. 1919.

<sup>2</sup>MIRANDE, M. Influence exercee par certaines vapeurs sur la cyanogenese vegetale. Procédé rapide pour la recherche des plants a acide cyanhydrique. Compt. Rend., 149:140-142. 1909.

<sup>3</sup>NOWOSAD, F. S., and MACVICAR, R. M. Adaptation of the "Picric acid test" method for selecting HCN free lines in sudan grass. Sci. Agr., 20:566-569. 1940.

amounts of the KCN alkaline picrate solution to eight test tubes (15 mm in diameter by 15 cm long):

Tube No.	Cc of solution
1	0.00
2	0.10
3	0.20
4	0.40
5	0.60
6	0.80
7	1.00
8	1.60

Bring the volume of each test tube up to 10 cc by adding distilled water. Stopper the tubes and keep them in a cool place. The number of milligrams of HCN present in each test tube is as follows: Tube 1, 0.00; tube 2, 0.005; tube 3, 0.01; tube 4, 0.02; tube 5, 0.03; tube 6, 0.04; tube 7, 0.05; and tube 8, 0.08. These standards keep satisfactorily for at least 2 weeks.

*Test paper.*—The test paper is prepared by cutting sheets of filter paper into strips 10 to 12 cm long and 0.5 cm wide and saturating them with alkaline picrate solution. The test papers should be moist when used.—P. G. HOGG, *Mississippi Agricultural Experiment Station, State College, Miss.*, and H. L. AHLGREN, *Wisconsin Agricultural Experiment Station, Madison, Wis.*

#### RATE OF GROWTH OF THE EMBRYO OF YOUNG BARLEY SEEDS ON EXCISED CULMS

It has been found possible to produce viable barley seeds on culms cut from the plant at pollination and allowed to continue their growth in distilled water.<sup>1</sup> Growth in length of the barley kernel is commonly nearly completed 9 to 10 days after pollination. The embryo, however, at ordinary temperatures continues to grow and differentiate rapidly for about another week. The attained size of barley embryos from spikes on excised culms possessing their three upper internodes and treated in the above manner was compared with those growing normally on the potted greenhouse plants.

A number of spikes of Manchuria barley, C.I. 2330, were emasculated and bagged. When the flowers were well opened, a few culms were cut off just beneath the third node below the spike and their cut ends placed in distilled water. These spikes, together with a number of receptive spikes remaining intact on the plants, were then pollinated by dusting and allowed to grow side by side on the greenhouse bench at Arlington Farm, Virginia. On the 9th, 10th, and 11th days after pollination samples of three or four kernels of each lot were killed and fixed. These were embedded in paraffin, sectioned sagittally, and stained. The lengths of the median sagittal sections

<sup>1</sup>POPE, MERRITT N. The production of barley seed through post-harvest pollination. *Jour. Heredity*, 26:411-413. 1935.

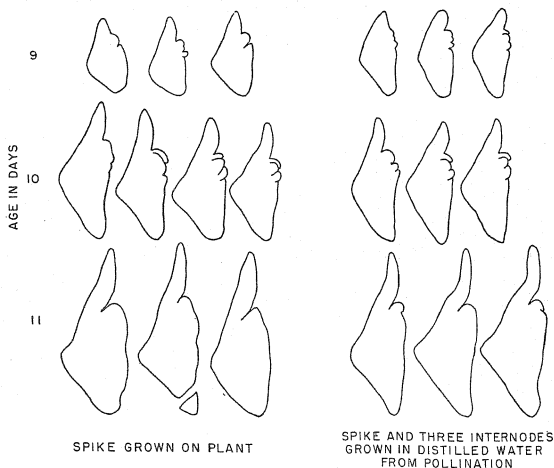


FIG. 1.—Median sagittal sections of Manchuria barley embryos from normal and excised culms of Manchuria barley.

were measured with an eyepiece micrometer and camera lucida drawings made of the harvested embryos. These measurements appear in Table 1 and the drawings in Fig. 1.

TABLE 1.—Length of embryos in mm from normal and excised culms of Manchuria barley.

Age in days after pollination	Embryo number				Average
	1	2	3	4	
From Normal Plants					
9	0.532	0.617	0.632	—	0.594
10	0.928	0.903	0.842	0.827	0.875
11	1.173	1.122	1.112	—	1.136
From Excised Culms					
9	0.612	0.617	0.653	—	0.627
10	0.847	0.831	0.847	—	0.842
11	1.112	1.127	1.158	—	1.132



In these experiments, growth of the barley embryo up to 11 days after pollination was as rapid and differentiation as far advanced on culms with three internodes attached cut from the plant at pollination and placed in distilled water as in culms growing normally on the plant.—MERRITT N. POPE, *Division of Cereal Crops and Diseases, U. S. Dept. of Agriculture, Washington, D. C.*

## BOOK REVIEWS

### TEXTBOOK OF BOTANY

*By E. N. Transeau, H. C. Sampson, and L. H. Tiffany. New York: Harper & Bros. XI + 812 pages, 7 plates, 424 figs. 1940.*

THE authors have produced a most modern textbook, embodying many of the recent great advances made by plant physiologists, plant pathologists, bacteriologists, geneticists, and ecologists, and giving far greater attention to physiological and ecological subjects than any other known texts in the field. A great deal of space is used to correct widely held but erroneous ideas concerning the physiological processes of plants.

A remarkably extended section (244 pages) in the first part of the volume is devoted to the physiology of plants. Following this, ample space is used for the conventional discussion of the principal organs of seed-bearing plants, but with a *physiological* emphasis. An interesting chapter deals with the origin of cultivated plants, while four chapters deal with heredity in plants. Much of the remainder of the book deals with a consideration of the great groups of plants, with the emphasis definitely on the economic and biological importance. For instance, the discussion of bacteria emphasizes the importance of the group with respect to sanitation and soil science; a treatment of plant diseases accompanies the chapter on fungi; and a consideration of "Under-water Environments" precedes the account of the algae. To such a degree is classification "subordinated" that nowhere do the authors give information that would enable the student to obtain a general outline of the main groups of plants. Phylogenetical considerations are omitted. Ecology, as a distinct subdivision of botany, is scarcely treated, and the word does not even appear in the index. Ecological considerations, nevertheless, are included throughout the entire subject matter.

The book is most attractively printed and bound and the many illustrations have been well chosen and are well reproduced. Typographical errors, or errors in references, are very few and relatively unimportant.

It is most probable that the greatest objections to the use of this work as a college textbook will come from that group of teachers who prefer a less extended treatment than is afforded by this 812-page survey. It is scarcely likely that it could be satisfactorily adapted to the requirements of a one semester course, but it would seem to be admirably suited to the needs of that steadily increasing body of instructors who wish to emphasize the whole range of living plant activities instead of the old-fashioned method of approach from the purely morphological or taxonomic viewpoint. (E. L. C.)

## STATISTICAL METHODS FOR RESEARCH WORKERS

By R. A. Fisher. *Edinburgh and London: Oliver and Boyd. Ed. 8. XIII + 344 pages, illus. 1941. 16 shillings.*

THE seven preceding editions of this well-known work have been reviewed in this JOURNAL so comparison with the seventh edition will be sufficient to guide statistical workers.

The entire text of the seventh edition and some new material is given in the new edition, but the number of pages has been reduced from 396 to 344 by slightly increasing the amount of print on each page. The additional material includes (1) methods of building up the analysis of variance, Section 24, one paragraph and Section 24.1, two paragraphs; (2), in Section 41, Example 39 is given a formula for the 0.1% point for comparison of intraclass correlations; (3) Section 49.3, The Precision of Estimated Scores, is new with the exception of the last paragraph which occurred in Section 49.2 of the previous edition; (4) the bibliography has been enlarged to include the period 1938 to 1941; and (5) the section numbers appear at the top of each page as a guide which is convenient in looking up sections.

The numbering of sections, tables, and examples found in the seventh edition have not been changed. The press work maintains the high standards found in the earlier editions. (F. Z. H.)

## AGRONOMIC AFFAIRS

## NEWS ITEMS

MRS. T. H. SHEN, Agronomist of the National Agricultural Research Bureau in China and Acting Head of the Wheat Improvement Department, died on October 7 in her laboratory at Yunchang, Szechuan, China. Her death is a great loss to scientific research in agriculture in China.

—A—

ROYSE P. MURPHY, formerly Assistant Professor, Division of Agronomy and Plant Genetics, University of Minnesota, was appointed Associate Professor of Agronomy at the Montana State College, Bozeman, Mont., effective February 1.

—A—

GEORGE M. GRANTHAM, Associate Professor and Research Associate in Soils at Michigan State College, has been retired due to ill health. Professor Grantham has been associated with the Michigan State College since the fall of 1914.

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No. 3

## SEED YIELDS OF VELVET BENT, *AGROSTIS CANINA* L., AS INFLUENCED BY THE KIND OF FERTILIZER APPLIED<sup>1</sup>

J. A. DeFRANCE AND T. E. ODLAND<sup>2</sup>

VELVET bent is becoming one of the most popular grasses for use as putting green turf on golf courses throughout the northern United States. It is also used for fine lawns and other types of grass areas. The production of velvet bent seed is a very recent development and the best conditions of soil fertility have not been known. Considerable velvet bent seed is now being produced in Rhode Island.

Very little experimental work has been reported with regard to the production of bent grass seed. North and Odland<sup>3</sup> studied the influence of different fertilizer mixtures on the yield of Rhode Island Colonial bent seed. The fertilizers consisted of various proportions of nitrogen, phosphoric acid, and potash. The ratios included in the test varied in the amounts of each fertilizer element applied. The yield of seed was influenced chiefly by the amount of nitrogen applied, whereas phosphorus and potash had little effect. With high applications of phosphoric acid and potash without a corresponding application of nitrogen, the yields were depressed. Lodging occurred with high applications of nitrogen. Time of maturity and weight per bushel of seed did not vary to any appreciable extent between the various treatments. Liming had only a slight tendency to increase the yields.

### MATERIALS AND METHODS

Seed of Piper velvet bent, produced the preceding year at the Rhode Island Agricultural Experiment Station, was planted in rows 1 foot apart on an area of the experimental plats on September 20, 1935. The rows were cultivated once with a wheelhoe during the last of October.

<sup>1</sup>Published by permission of the Director of Research, Rhode Island Agricultural Experiment Station, Kingston, R. I., as contribution No. 605. Received for publication July 25, 1941.

<sup>2</sup>Associate Agronomist and Agronomist, respectively.

<sup>3</sup>NORTH, H. F. A., and ODLAND, T. E. Seed yields of Rhode Island colonial bent (*Agrostis tenuis* Sibth) as influenced by the kind of fertilizer applied. Jour. Amer. Soc. Agron., 26:939-945. 1934.

The area was divided into plats, each 11 feet 4 inches by 22 feet, separated by borders 3 feet wide. The plats were in random arrangement and replicated four times. Fertilizer was applied in two doses, two weeks apart in the early spring of each succeeding year. The various ratios of nitrogen, phosphoric acid, and potash applied are shown in the following tables. Each unit of a given ratio represents an application of 10 pounds per acre of nitrogen, phosphoric acid, or potash annually.

The soil where the experiment was conducted is a uniform silt loam underlain with gravel. A composite soil sample taken in 1935 showed an acidity of pH 4.5.

## RESULTS

Data on stand, height, percentage of lodging, seed weight, and ratio of hay to seed are presented in Table 1. The notes on percentage of stand show a response in favor of the higher rate of application of nitrogen. Additional nitrogen, phosphorus, or potash had no consistent effect on increasing height. Increasing the amounts of nitrogen was found to increase the percentage of lodging.

TABLE 1.—The average growth and production of *Piper velvet bent* grown with different ratios of nitrogen, phosphoric acid, and potash for period of 1936-39.

Fertilizer ratio	Stand %	Height, inches	Lodging % <sup>a</sup>	Ratio of seed to hay	Seed weight, lbs. per bu.
0-0-0.....	77	17.6	0.0	1:25	27
2-6-3.....	88	20.2	0.5	1:27	27
4-6-3.....	89	20.0	1.0	1:33	26
6-6-3.....	92	20.5	3.7	1:31	25
6-2-3.....	89	19.4	3.3	1:35	25
6-10-3.....	93	20.2	4.7	1:31	25
6-6-1.....	94	20.2	6.0	1:26	25

<sup>a</sup>No lodging occurred in 1939.

It is interesting to note that the weight per bushel of cleaned seed had a tendency to decrease with additional applications of nitrogen. On plats that received 20, 40, and 60 pounds of nitrogen per acre there was a progressive decrease in weight per bushel.

TABLE 2.—Yields of cleaned seed of *Piper velvet bent* in pounds per acre with different ratios of nitrogen, phosphoric acid, and potash.

Fertilizer ratio	Annual application, lbs. per acre <sup>a</sup>			Cleaned seed in lbs. per acre			
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	1937	1938	1939	Average
0-0-0...	0	0	0	53.8±3.8	74.2±4.6	51.2±4.0	59.7±2.4
2-6-3...	20	60	30	53.5±3.8	98.3±6.1	66.3±5.1	72.7±2.9
4-6-3...	40	60	30	55.7±3.9	98.7±6.1	82.5±6.4	79.0±3.2
6-6-3...	60	60	30	55.7±3.9	119.0±7.3	109.5±8.5	94.7±3.9
6-2-3...	60	20	30	50.0±3.5	118.0±7.3	96.7±7.5	88.2±3.7
6-10-3...	60	100	30	61.7±4.4	130.0±8.0	135.7±10.5	109.1±4.6
6-6-1...	60	60	10	77.0±5.5	136.0±8.4	133.3±10.3	115.4±4.8

<sup>a</sup>Nitrogen from sulfate of ammonia, phosphoric acid from superphosphate, and potash from muriate of potash. Probable errors obtained by use of the deviation from the mean method.

The seed yields obtained with the various fertilizer ratios applied are presented in Table 2. The figures show nitrogen has been the most effective nutrient in increasing yields. The seed yield has been largely in proportion to the amount of nitrogen applied. The average yield for the three seasons ranged from 60 pounds per acre on the check plots to 109 and 115 pounds where the 6-10-3 and 6-6-1 ratios were applied. The highest average yield per acre for any one year was 136 pounds. This occurred with the ratios 6-6-1 in 1938 and 6-10-3 in 1939. The variation in the amount of potash applied had no consistent effect on the yield of seed. However, by increasing the amount of phosphoric acid to 10 units an increase in yield was obtained.

In Table 3, the differences in average yields between various treatments have been arranged in a convenient way for ready comparison. The increase or decrease in seed yields obtained with the fertilizer ratio shown in the horizontal row at the top of the table as compared with any ratio shown in the column at the left of the table may be obtained from these figures.

The yields of air-dry hay in pounds per acre and the ratio of seed to hay are shown in Table 4. The yield of hay was increased by the addition of nitrogen in the fertilizer. The addition of two units of potash to the 6-6-1 ratio had no consistent effect on increasing hay yields. Likewise the addition of four units of phosphoric acid thus increasing the 6-2-3 ratio to a 6-6-3 ratio did not increase the yields. However, by adding eight units of phosphoric acid to the 6-2-3 and four units to the 6-6-3 ratios, making a 6-10-3 ratio, an increase in yield was obtained in all cases. A yield of over 2 tons per acre was harvested from these high phosphorus plots in 1937. In the following year the same plots maintained the highest average yield of hay, although the yields of all different fertilizer ratio plots in general decreased. This was accompanied by an increase in ratio of seed to hay.

No correlation between the amount of seed produced and the yield of hay was apparent. The highest ratio of seed to hay occurred on the 6-6-1 plots in 1939 when it reached 1 : 14.

In a series of tests to determine the effect of applications of limestone at the rate of 1 ton per acre in 1938 and 1939 on seed production of Piper velvet bent, it was found that small increases in yield were obtained on all the different fertilizer ratio plots where limestone was applied. The unlimed plots had a pH of 4.26 compared to 4.80 on the limed areas.

## DISCUSSION

With regard to the effects of nitrogen and potash, in general, the results of this study conform closely with those reported previously on the influence of different fertilizers on seed yields of Rhode Island colonial bent. However, there was more response obtained by increasing the phosphoric acid in the ratios on velvet bent than on Rhode Island bent. The average yields of Rhode Island bent were somewhat larger on the high-nitrogen plots than the yields of velvet bent. However, the treatments were applied at the rate of 1,500 pounds per acre compared with 1,000 pounds in this test.

The area was divided into plats, each 11 feet 4 inches by 22 feet, separated by borders 3 feet wide. The plats were in random arrangement and replicated four times. Fertilizer was applied in two doses, two weeks apart in the early spring of each succeeding year. The various ratios of nitrogen, phosphoric acid, and potash applied are shown in the following tables. Each unit of a given ratio represents an application of 10 pounds per acre of nitrogen, phosphoric acid, or potash annually.

The soil where the experiment was conducted is a uniform silt loam underlain with gravel. A composite soil sample taken in 1935 showed an acidity of pH 4.5.

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Data on stand, height, percentage of lodging, seed weight, and ratio of hay to seed are presented in Table 1. The notes on percentage of stand show a response in favor of the higher rate of application of nitrogen. Additional nitrogen, phosphorus, or potash had no consistent effect on increasing height. Increasing the amounts of nitrogen was found to increase the percentage of lodging.

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Fertilizer ratio	Stand %	Height, inches	Lodging, %	Ratio of seed to hay	Seed weight, lbs. per bu.
0-0-0.....	77	17.6	0.0	1:25	27
2-6-3.....	88	20.2	0.5	1:27	27
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\*No lodging occurred in 1930.

It is interesting to note that the weight per bushel of cleaned seed had a tendency to decrease with additional applications of nitrogen. On plats that received 20, 40, and 60 pounds of nitrogen per acre there was a progressive decrease in weight per bushel.

TABLE 2.—*Yields of cleaned seed of Piper velvet bent in pounds per acre with different ratios of nitrogen, phosphoric acid, and potash.*

Fertilizer ratio	Annual application, lbs. per acre <sup>1</sup>			Cleaned seed in lbs. per acre			
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	1937	1938	1939	Average
0-0-0...	0	0	0	53.8±3.8	74.2±4.6	51.2±4.0	59.7±2.4
2-6-3...	20	60	30	53.5±3.8	98.3±6.1	66.3±5.1	72.7±2.9
4-6-3...	40	60	30	55.7±3.9	98.7±6.1	82.5±6.4	79.0±3.2
6-6-3...	60	60	30	55.7±3.9	119.0±7.3	109.5±8.5	94.7±3.9
6-2-3...	60	20	30	50.0±3.5	118.0±7.3	96.7±7.5	88.2±3.7
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The area was divided into plots, each 11 feet 4 inches by 22 feet, separated by borders 3 feet wide. The plots were in random arrangement and replicated four times. Fertilizer was applied in two doses, two weeks apart in the early spring of each succeeding year. The various ratios of nitrogen, phosphoric acid, and potash applied are shown in the following tables. Each unit of a given ratio represents an application of 10 pounds per acre of nitrogen, phosphoric acid, or potash annually.

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It is interesting to note that the weight per bushel of cleaned seed had a tendency to decrease with additional applications of nitrogen. On plots that received 20, 40, and 60 pounds of nitrogen per acre there was a progressive decrease in weight per bushel.

TABLE 2.—Yields of cleaned seed of *Piper velvet bent* in pounds per acre with different ratios of nitrogen, phosphoric acid, and potash.

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4-6-3...	40	60	30	55.7±3.9	98.7±6.1	82.5±6.4	79.0±3.2
6-6-3...	60	60	30	55.7±3.9	119.0±7.3	109.5±8.5	94.7±3.9
6-2-3...	60	20	30	50.0±3.5	118.0±7.3	96.7±7.5	88.2±3.7
6-10-3...	60	100	30	61.7±4.4	130.0±8.0	135.7±10.5	109.1±4.6
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TABLE 3.—Increase or decrease between average yields of *Piper velut bent* seed under various fertilizer treatments.

Fertilizer ratio	Cleaned seed in lbs. per acre for ratios of						Average yield, 1937-39, lbs. per acre
	0-0-0	2-6-3	4-6-3	6-6-3	6-2-3	6-10-3	6-6-1
0-0-0	0	13.0±3.8	19.3±4.0	35.0±4.6	28.5±4.4	49.4±5.2	59.7±2.4
2-6-3	—	0	6.3±4.3	22.0±4.9	15.3±4.7	36.4±5.4	72.7±2.9
4-6-3	—	—	0	15.7±5.0	9.3±4.9	30.1±3.6	79.0±3.2
6-6-3	—	—	—	0	-6.5±5.4	14.4±6.0	94.7±3.9
6-2-3	—	—	—	—	0	20.9±5.9	88.2±3.7
6-10-3	—	—	—	—	—	0	109.1±4.6
6-6-1	—	—	—	—	—	—	115.4±4.8

TABLE 4.—*Air-dry hay yields of Piper velvet bent and the ratio of seed to hay, 3-year average.*

Fertilizer ratio	Hay yields in lbs. per acre				Ratio of seed to hay			
	1937	1938	1939	Average	1937	1938	1939	Average
0-0-0....	1,820	1,435	1,172	1,476	1:34	1:19	1:23	1:25
2-6-3....	2,292	2,187	1,435	1,971	1:43	1:22	1:22	1:27
4-6-3....	3,185	2,677	1,872	2,578	1:57	1:27	1:23	1:33
6-6-3....	3,797	3,150	1,925	2,957	1:68	1:26	1:18	1:31
6-2-3....	3,745	3,517	1,872	3,045	1:75	1:30	1:19	1:35
6-10-3...	4,077	3,867	2,292	3,412	1:66	1:30	1:17	1:31
6-6-1....	3,937	3,185	1,890	3,004	1:51	1:23	1:14	1:26

Each succeeding year the yield of hay decreased on all the different fertilizer ratio plots. Associated with this there was a gradual narrowing of the ratio of seed to hay. This is a favorable factor when seed production is paramount.

Over the 3-year period and under the conditions of this test, medium proportions of phosphorus and small proportions of potash in the fertilizer appeared sufficient for production of Piper velvet bent seed. The high level of phosphorus, however, produced the highest yield during the third year of the test.

A fairly consistent increase in yield of seed resulted with increasing amounts of nitrogen applied. There is a limit, however to the amount of nitrogen that can be applied because of the danger of lodging and a consequent reduction in the yield of seed due to the difficulty of harvesting.

Based on this study, it appears that a fertilizer high in nitrogen, such as a 10-5-5, 10-6-4, 8-6-4, or an equivalent, applied at the rate of 600 to 800 pounds per acre should be sufficient for fertilizing velvet bent for seed production.

An interesting observation on the effect of fertilizer applications on bent grass for seed production has been made on two occasions about 10 years apart when outbreaks of army worms occurred. At both of these times the army worm caused extensive damage to newly seeded areas that had been treated with a heavy application of fertilizer before seeding. The fertilizer helped develop soft, tender plants and foliage that were very palatable to the army worms. As a consequence, the first year's stand was devoured and the crop was a total loss. Adjacent seed-producing areas that were 2, 3, or 4 years old were not so attractive to the worms and very little damage was done. From these experiences it appears necessary to avoid heavy applications of fertilizer as a pre-seeding treatment and to take early precautionary measures to control the army worms if an outbreak occurs.

#### SUMMARY

A 3-year study was made of the effect of different fertilizer ratios on seed production of Piper velvet bent. The fertilizers consisted of various proportions of inorganic nitrogen, phosphoric acid, and

potash. The following ratios were used in the test and were applied at the rate of 1,000 pounds per acre: 2-6-3, 4-6-3, 6-6-3, 6-2-3, 6-10-3, and 6-6-1. There was also a no-fertilizer treatment.

By increasing the amounts of nitrogen in the fertilizer ratio the yield of seed was increased. A small amount of lodging occurred with the highest applications of nitrogen. Medium proportions of phosphorus and small proportions of potash in the fertilizer were sufficient for the production of seed.

Yields of hay decreased in succeeding years with a consequent narrowing of the ratio of seed to hay. The yield of hay was increased by additional units of nitrogen supplied in the fertilizer. The addition of two units of potash had no consistent effect on increasing hay yields. High phosphoric acid resulted in an increase.

The higher ratios of nitrogen applied resulted in an increase in percentage of stand and also in the amount of lodging. The average height of plants was little affected by the type of fertilizer used.

Weight per bushel of cleaned seed was slightly decreased with increased applications of nitrogen.

An annual application of 600 to 800 pounds per acre of a fertilizer such as a 10-5-5, 10-6-4, or 8-6-4 is suggested for use in velvet bent seed production under these conditions.

## INHERITANCE OF REDUCED LATERAL SPIKELET APPENDAGES IN THE NUDIHAXTONI VARIETY OF BARLEY<sup>1</sup>

WARREN H. LEONARD<sup>2</sup>

THE inheritance of reduced lateral spikelet appendages in varieties of barley classified in the *Hordeum intermedium* species offers a problem of considerable interest.

Harlan (6)<sup>3</sup> has recognized four species of cultivated barley on the basis of the fertility of the lateral spikelets, namely, *H. vulgare*, *H. intermedium*, *H. distichon*, and *H. deficiens*. The species *H. intermedium* is one in which at least some of the lateral spikelets are fertile. The lemmas of these spikelets bear neither awns nor hoods. Some varieties of this species breed true for fully fertile lateral spikelets, while others are homozygous for only partial fertility of these spikelets. Nudihaxtoni is a variety with all spikelets fertile, but with awnless lateral spikelets.

The results of a study on the relation of fertility of the lateral spikelets to reduced appendages (awns or hoods) on these spikelets are presented in this paper.

### LITERATURE REVIEW

Fertility of the lateral spikelets in relation to awn development was investigated in India by Bose, *et al.* (1). They crossed Pusa Type 21, a 6-rowed variety (*H. vulgare* var. *pallidum*), and Pusa Type 1, a 2-rowed variety (*H. distichon*). The F<sub>1</sub> was intermediate in fertility and awn development. The cross was carried through the F<sub>3</sub> generation. These workers concluded that "fertility of the lateral florets and development of awns on their outer palea have been observed to be perfectly linked and their inheritance has been found to depend on the interaction of the same genetic factors." The segregation in F<sub>2</sub> for fertility of the lateral spikelets and of awn development on the lemmas of the lateral florets gave a good fit to a 1:2:1 ratio, that is, 2-rowed, intermediate, and 6-rowed forms. The lemmas of the lateral spikelets were fully awned on the 6-rowed, awn-tipped on the intermediate type, and with no awn tips on the 2-rowed.

The inheritance of the awnless condition of the lateral spikelets was studied by Miyake and Imai (9). Some varieties entirely lack awns on the lateral spikelets. When these were crossed with normal ones (presumably normal 6-rowed), the growth of the awns of the lateral spikelets was found to be imperfect or intermediate in character in the F<sub>1</sub> generation. In the next generation, Miyake and Imai observed a 1:2:1 segregation for perfect awns, intermediate awns, and

<sup>1</sup>This material is taken from a thesis presented to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy. The data all were obtained at the Colorado Experiment Station at Fort Collins, Colo. Published with the approval of the Director of the Experiment Station as Scientific Series Paper No. 129. Received for publication October 15, 1941.

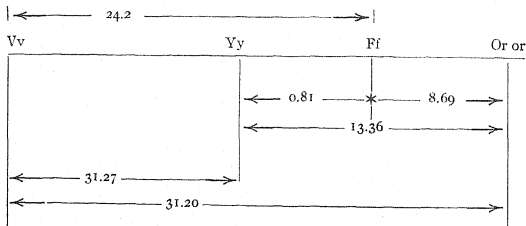
<sup>2</sup>The writer wishes to express his appreciation for helpful suggestions received during the course of this study from Dr. F. R. Immer, Professor of Agronomy and Plant Genetics, University of Minnesota, and from Dr. D. W. Robertson, Agronomist, Colorado Experiment Station.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 220.

awnless for the lateral spikelets. The genetic character of awned and awnless was expressed on the basis of a single-factor difference. These workers also find a short-awned condition on the lemmas of the lateral spikelets. The various conditions of long-awned, short-awned, and awnless were explained on the basis of an allelic series.

Glinyany (5) crossed Nudihaxtoni with a 6-rowed, hooded variety (*II. vulgare* var. *trifurcatum*). The Nudihaxtoni parent had long awns on the central spikelets and "awnlike appendages" on the lateral spikelets. In the  $F_2$  generation, he was able to divide the awned plants into three groups, the ratio being 1 : 2 : 1. The ratio of hooded and intermediate plants to awned ones was 3 : 1. His explanation is as follows: "On the basis of the data obtained, the conclusion must be drawn that Nudihaxtoni and Trifurcatum, as regards awns and furcas (hoods), differ in two pairs of factors. Nudihaxtoni has the genotype *aaNN* and Trifurcatum *AAnn*. The factor *a* promotes the development of awns on all spikelets; the factor *N* awns on only the central spikelets, inhibiting their development on lateral ones; hence, the awned character of the form Nudihaxtoni. The appearance of furcas (hoods) is determined by the single factor *A*, which is an allelomorph of the factor *a* and is dominant over it. The factor *n* promotes the development of awns on all spikelets, the factor *A* being epistatic to it. The dominance of *N* over *n* is incomplete."

Seven linkage groups have been established in cultivated barley, the literature for which has been reviewed by Robertson, Wiebe, and Immer (13). Because the factor pair concerned in this study was found to be located in group I, the literature will be reviewed only for that group. The green vs. chlorina (*Ff*) factor pair in Minnesota 84-7 was located in group I by Robertson, *et al.* (11). It was linked with the non-6-row vs. 6-row (*Vv*) factor pair with a recombination value of  $18.3 \pm 0.74\%$ . This linkage was based on  $F_2$  data. Recently, Robertson and Coleman (12) have located two other factor pairs for chlorophyll-deficient seedlings in group I. These are green vs. orange seedling color (*Or or*) found in Trebi IV and green vs. virescent seedling color (*Yy*) found in Minnesota 72-8. The recombination value between non-6-row vs. 6-row (*Vv*) and green vs. chlorina (*Ff*) for a Trebi IV  $\times$  Minnesota 84-7 cross was  $24.2\%$  as determined from  $F_3$  data. These genes were located in group I as follows:



#### MATERIALS AND METHODS

This study on the inheritance of reduced lateral spikelet appendages in Nudihaxtoni was carried on at the Colorado Experiment Station from 1931 to 1940, inclusive.

## SYMBOLS FOR GENETIC CHARACTERS

The reduced lateral spikelet appendage condition was investigated in relation to the factor pairs for non-6-row vs. 6-row (Vv) and intermedium vs. non-intermedium (Ii). The factor pairs listed below were involved in various crosses, the standard nomenclature suggested by Robertson, Wiebe, and Immer (13) being followed:

Linkage group	Character pair	Symbols
I	Non-6-row vs. 6-row Green vs. chlorina seedling color Green vs. orange seedling color	Vv Ff Or or
II	Black vs. white floral bracts	Bb
III	Covered vs. naked caryopsis	Nn
IV	Hoods vs. awns Intermedium versus non-intermedium	Kk Ii
V	Long vs. short-haired rachillas	Ss
VI	Green vs. xantha seedling color	X <sub>c</sub> x <sub>c</sub>
VII	Green vs. virescent seedling color	F <sub>e</sub> f <sub>e</sub>

## DESCRIPTION OF VARIETIES

The barley strains used in crosses were Nudihaxtoni, Colsess IV, Colsess V, Antrum, Minnesota 84-7, Nigrilaxum, and Nigrinudum. These lines were made available either by the Colorado Experiment Station or the U. S. Dept. of Agriculture.

*H. intermedium nudihaxtoni* (C. I. 2213) is an intermedium variety with complete fertility of the lateral spikelets. It is a naked barley with white kernels and floral bracts. The lemmas of the central spikelets are long-awned, while those of the lateral spikelets are awnless (Fig. 1).

A factorial description of Nudihaxtoni, together with the lines crossed with it, is given in Table 1.

TABLE 1.—List of the factor pairs found in the different varieties.

Variety	Symbols for characters									
	Vv	Ff	Or or	Bb	Nn	Kk	Ii	Ss	X <sub>c</sub> x <sub>c</sub>	F <sub>e</sub> f <sub>e</sub>
Nudihaxtoni.....	vv	FF	Or Or	bb	nn	kk	II	SS	X <sub>c</sub> X <sub>c</sub>	F <sub>e</sub> F <sub>e</sub>
Colsess IV.....	vv	FF	Or Or	bb	NN	KK	II	ss	X <sub>c</sub> x <sub>c</sub>	F <sub>e</sub> F <sub>e</sub>
Colsess V.....	vv	FF	Or Or	bb	NN	KK	II	ss	X <sub>c</sub> X <sub>c</sub>	f <sub>e</sub> f <sub>e</sub>
Antrum.....	vv	FF	Or Or	BB	—	KK	—	—	X <sub>c</sub> X <sub>c</sub>	F <sub>e</sub> F <sub>e</sub>
Minnesota 84-7*	vv	ff	Or or	bb	NN	kk	—	SS	X <sub>c</sub> x <sub>c</sub>	—
Nigrilaxum.....	VV	FF	Or Or	BB	nn	KK	II	—	X <sub>c</sub> X <sub>c</sub>	F <sub>e</sub> F <sub>e</sub>
Nigrinudum.....	VV	FF	Or Or	BB	nn	kk	II	ss	X <sub>c</sub> X <sub>c</sub>	F <sub>e</sub> F <sub>e</sub>

\*A 6-row segregate which carried the Or or factor pair was used.



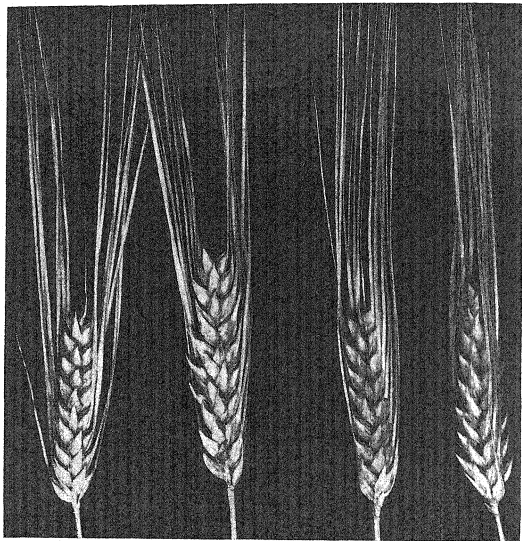


FIG. 1.—Heads of the Nudi-haxtoni parent.

#### EXPERIMENTAL METHODS

The experimental methods used were similar to those followed by Robertson (10). In most instances, a random sample of the  $F_2$  plants was carried to the  $F_3$  generation to determine the  $F_2$  genotypes. A few heads were threshed from each  $F_2$  plant, the seed of which was planted in individual 8-foot rows for the  $F_3$  segregation.

The observed data were compared to calculated theoretical ratios and tested for goodness of fit by the use of  $X^2$  when two or more phenotypic classes were involved (8). The interpretation of the significance of  $X^2$  values was the same as that used by Fisher (3).

The linkage determinations from  $F_2$  data were made by use of the Collins (2) formula, which is as follows:  $p = \sqrt{\frac{AB - 2Ab}{AB + Ab}} \pm \sqrt{\frac{(1-p^2)(2+p^2)}{3Np^2}}$ , where  $p$  = the recombination percentage in the coupling phase. This formula satisfies the conditions of maximum likelihood. The standard error for this formula was worked out by Immer (7). The  $F_2$  and  $F_3$  data were combined for the determination of the recombination value by the method suggested by Immer (7) as modified by Robertson and Coleman (12).

## EXPERIMENTAL RESULTS

The constitution of Nudihaxtoni for the non-6-row vs. 6-row (Vv) and for the intermedium vs. nonintermedium (Ii) factor pairs was determined as a preliminary step. Leonard (8) found Nigrilaxum to be VVII in constitution. Nudihaxtoni was crossed with Nigrilaxum, the progeny being carried to the  $F_2$  generation to determine the  $F_2$  genotypes. The data are given in Table 2. The data afford a good fit to the calculated 1:2:1 ratio for a single-factor difference. The genetic constitution of Nudihaxtoni appears to be vvII, that is, it is a 6-row barley.

TABLE 2.—Observed and calculated  $F_2$  genotypes for non-6-row vs. 6-row as determined by the  $F_2$  segregation in a Nudihaxtoni  $\times$  Nigrilaxum cross.

Item	Number of indicated genotype					
	2-row (VV)	Inter- mediate (Vv)	6-row (vv)	Total	$\chi^2$	P
Observed counts.....	295	547	294	1,136	—	—
Calculated 1:2:1 ratio.	284	568	284	1,136	1.5546	0.4742

## REDUCED LATERAL SPIKELET APPENDAGES

The inheritance of normal vs. reduced lateral spikelet appendages (hoods or awns) was studied in crosses between Nudihaxtoni and varieties of *H. vulgare*, namely, Colseß IV, Colseß V, and Antrum. The  $F_1$  plants were typically 6-rowed and hooded, although the hoods on the lateral spikelets were not fully developed. The  $F_2$  plants segregated for normal and reduced lateral spikelet appendages. The normal plants had fully developed awns or hoods on all lemmas, while the reduced group was characterized by the absence of appendages on the lemmas of the lateral spikelets. The intermediate (heterozygous) class, which was grouped with the normals, was difficult to separate from the normals. Hooded intermediates had hoods on the lateral spikelets which were reduced in size, often having a single instead of a trifurcate appendage. The awned intermediates were those plants with awns half or less than half the lengths of the ones on the lemmas of the central spikelets. The inheritance of the normal vs. reduced (Lr, lr) characteristic was studied in the Nudihaxtoni  $\times$  Colseß IV, Nudihaxtoni  $\times$  Colseß V, and Antrum  $\times$  Nudihaxtoni crosses (Fig. 2).

Since the  $F_2$  material was difficult to classify, the selfed  $F_2$  plants were analyzed in the  $F_3$  generation, the data for which appear in Table 3. The observed  $F_3$  data afford a good fit to the calculated 1:2:1 ratio for normal vs. intermediate vs. reduced lateral spikelet appendages. Thus, there is an indication that the normal vs. reduced (Lr, lr) characteristic is due to a single-factor difference, the normal condition being dominant over reduced since the  $F_1$  plants have appendages on the lateral spikelets.

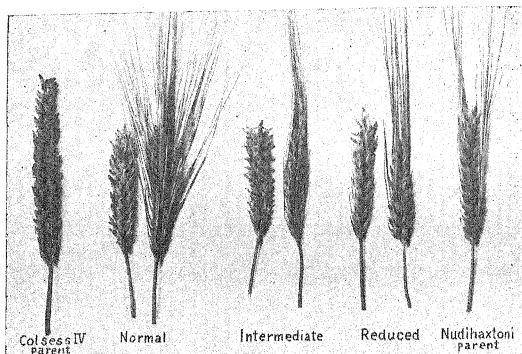


FIG. 2.—Normal, intermediate, and reduced  $F_2$  classes in a Colseess IV  $\times$  Nudihaxtoni cross.

TABLE 3.—Observed and calculated  $F_2$  genotypes for normal vs. reduced lateral spikelet appendages as determined from the  $F_3$  segregation in crosses of Nudihaxtoni with three 6-row types.

Cross	Number of indicated genotype*					
	Normal	Inter- me- diates	Re- duced	Total	$\chi^2$	P
Nudihaxtoni $\times$ Colseess IV.....	480	915	420	1,815	4.0909	0.1305
Colseess V $\times$ Nudihaxtoni.....	274	540	240	1,054	2.8349	0.2470
Antrum $\times$ Nudihaxtoni.....	153	341	161	655	1.3084	0.5329

\*Compared with a calculated 1:2:1 ratio.

The cross between 6-rowed Nudihaxtoni (vv) and 2-rowed Nigri-laxum (VV) was studied in relation to the normal vs. reduced appendage (Lr, lr) factor pair. A separation was made in the  $F_2$  for reduced vs. slightly tipped lateral spikelet appendages, but the  $F_3$  segregation showed this classification to be unjustified. The slight tipping found in the  $F_2$  was reconciled with the variation that occurs in the Nudihaxtoni parent in this regard. Apparently, the factor for normal (Lr, Lr) expresses itself only in the presence of the factor for 6-row (Fig. 1). Similar results were obtained for a Nudihaxtoni  $\times$  Nigri-nudum cross, although the progeny were not carried into the  $F_3$  generation in this case.

The data suggest that Nudihaxtoni is really a 6-rowed barley (vvII) with a factor for appendage reduction on the lateral spikelets (lr, lr). Gillis (4) first mentioned this possibility in some intermedium barleys, having observed that *H. intermedium cornutum* differed from ordinary 6-rowed only in the matter of no hoods being carried on the lateral spikelets.

#### INTERRELATIONSHIPS OF REDUCED LATERAL SPIKELET APPENDAGES WITH OTHER CHARACTERS

The factor pair for normal vs. reduced lateral spikelet appendages (Lr, lr) was studied in Nudihaxtoni in relation to various factor pairs in known linkage groups. Since the normal condition (Lr, Lr) can occur only in 6-rowed varieties, it early became apparent that 6-row linkage testers were necessary in all cases. The normal spikelet appendage condition (Lr, Lr) has not been found to act in the 2-rowed barleys studied.

*Characters inherited independently.*—The various characters inherited independently of the normal vs. reduced (Lr, lr) factor pair are given in Table 4. While the  $F_1$  plants had fully fertile lateral spikelets, the appendages were intermediate between fully awned or hooded and the reduced condition. Since the normal and reduced classes were difficult to separate in the  $F_2$  generation, the selfed  $F_2$  plants were grown in the  $F_3$  generation to verify the  $F_2$  genotypes.

The observed segregations in the  $F_3$  generation indicate good fits in all cases to the calculated ratios for independent inheritance. The normal vs. reduced lateral spikelet appendage (Lr, lr) factor pair appears to be inherited independently of the factor pairs for black vs. white floral bracts (Bb), covered vs. naked (Nn), hoods vs. awns (Kk), long- vs. short-haired rachillas (Ss), green vs. xantha seedling color ( $X_c x_c$ ), and green vs. chlorina ( $F_c f_c$ ). The normal vs. reduced (Lr, lr) factor pair is evidently inherited independently of factor pairs known to be located in linkage groups II, III, IV, V, VI, and VII.

*Linkage of characters.*—The interrelationship of normal vs. reduced lateral spikelet appendages (Lr, lr) and green vs. orange seedling color (Or, or) was studied in a cross between Nudihaxtoni and a 6-rowed form of Minnesota 84-7. The green vs. orange (Or, or) factor pair was found by Robertson and Coleman (12) to be located in linkage group I. Minnesota 84-7 was also homozygous for chlorina (ff) located in the same group. To study the linkage relations, the chlorina plants were included with the green ones. The mature plants were classified in  $F_3$  for normal vs. reduced lateral spikelet appendages (Lr, lr), the data for which are given in Table 5.

The data indicate a poor fit to the calculated 9:3:4 ratio for independent inheritance. It is noted that the normal green plants (recombinations) are fewer than would be expected for independent inheritance, while the green plants with reduced appendages are greater than expected. Linkage in the repulsion phase is indicated. The Collins formula was used to determine the recombination value, since the aB and ab classes are indistinguishable. A recombination percentage of 27.82 with a standard error of 7.74 was obtained. The

TABLE 4.—Observed  $F_2$  genotypes for normal vs. reduced lateral spikelet appendages and factors inherited independently as determined by  $F_3$  segregations in crosses of *Nudihaxtoni* with other varieties.

Nudihaxtoni crossed with	Geno- type studied	Link- age group	Genotypes*										Total	X <sup>2</sup>	P
			AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb				
Antrum . . . . .	LrlrBb	II	42	71	40	92	164	85	40	75	46	655	4.0908	0.8481	
Colsess IV . . . . .	LrlrNn	III	123	229	127	215	452	249	102	223	95	1,815	9.4500	0.3075	
Colsess IV . . . . .	LrlrKk	IV	126	230	123	230	446	239	118	212	91	1,815	8.6602	0.3733	
Colsess V . . . . .	LrlrKk	IV	83	128	63	142	279	119	60	129	51	1,054	11.5719	0.1728	
Colsess IV . . . . .	LrlrSs	V	85	136	61	135	252	129	65	125	69	1,057	7.1670	0.5194	
Colsess IV . . . . .	LrlrX <sub>6</sub> <sup>ex</sup>	VI	106	175	—	173	344	—	78	181	—	1,057	5.1935	0.3947	
Colsess V . . . . .	LrlrF <sub>6</sub> <sup>ex</sup>	VII	63	135	76	141	275	124	63	123	54	1,054	6.2154	0.6234	

\*Compared with a calculated 1:2:1:2:4:2:1:1:1 ratio in all cases except for the LrlrX<sup>ex</sup><sub>6</sub> genotypes for which a 1:1:2:4:1:1:2 ratio was used.

TABLE 5.—The  $F_2$  segregation of normal vs. reduced and green vs. orange in a *Nudihaxtoni*  $\times$  *Minnesota 84-7* (6-row) cross.

Item	Number of plants with characters indicated					
	Green*		Orange	Total	X <sup>2</sup>	P
	Normal	Reduced				
Observed count.....	698	310	370	1,378		
Calc. 9:3:4 ratio.....	775.12	258.38	344.50	1,378	19.8733	V. small
Calc. 27.82% recombination.....	715.66	317.84	344.50	1,378	2.5167	0.2931

\*Includes chlorina plants.

observed data afforded a good fit to the calculated segregation for 27.82% of recombination. Thus, the factor pair for normal vs. reduced lateral spikelet appendages (Lr, lr) appears to be located in linkage group I.

The genotypic constitution of the classes that survived in the  $F_2$  was determined from the  $F_3$  segregation of selfed  $F_2$  plants. The linkage intensity was computed for the  $F_3$  genotypes. The recombination value was found to be  $38.58 \pm 1.20\%$ . The observed  $F_3$  data for the normal vs. reduced (Lr, lr) and green vs. orange seedlings (Or, or) are given in Table 6. The data afford a good fit to the calculated ratio for 38.58% of recombination. The data for the  $F_2$  genotypes as determined from the  $F_3$  segregation corroborate the evidence of linkage of the Lrlr and Oror factor pairs as indicated from  $F_2$  phenotypes. It should be noted that there is a large discrepancy in the recombination values as determined in this case from  $F_2$  and  $F_3$  data. This is obviously due to the small amount of information obtained from the  $F_2$  phenotypes when only the AB and Ab classes can be used.

TABLE 6.—Observed and calculated  $F_3$  genotypes as determined from the  $F_3$  segregation in a *Minnesota 84-7*  $\times$  *Nudihaxtoni* cross.

Item	Number of indicated genotype								
	Normal (LrLr)		Heterozy- gotes (LrIr)		Reduced (lrlr)		To- tal	X <sup>2</sup>	P
	OrOr	Oror	OrOr	Oror	OrOr	Oror			
Observed count. . . .	53	153	160	346	136	160	1,008	—	—
Calc. 1:2:2:4:1:2 ratio. . . . .	84	168	168	336	84	168	1,008	19.9346	0.0013
Calc. 38.58 pct. recombination. . .	50.01	159.24	159.24	353.53	126.74	159.23	1,008	1.2676	0.9322

## DISCUSSION OF RESULTS

The *H. intermedium nudihaxtoni* variety appears to be a true 6-row barley, so far as the factors for rows and intermedium are concerned, being vvII in constitution. In addition, there is a factor present for the reduction of the appendages on the lemmas of the lateral spikelets, whether awns or hoods. This factor pair has been designated as normal vs. reduced lateral spikelet appendages (Lr, lr). Ordinary 6-row varieties appear to carry this factor in the dominant condition (Lr, Lr), while in a variety like Nudihaxtoni it is recessive (lr, lr). The normal vs. reduced condition appears to be due to a single-factor difference. Apparently it is similar to one found by Miyake and Imai (9). They describe the condition as awnless vs. awned lateral florets, but did not recognize that the same factor pair, or one similar to it, might also reduce the hoods on the lateral spikelets. Glinyany (5) worked with Nudihaxtoni and found the same factor pair reported in this study. In neither case did these investigators make clear the relationship of this factor pair to the non-6-row vs. 6-row (Vv) and intermedium vs. non-intermedium (Ii) factor pairs.

The interrelationships of the factor pair for normal vs. reduced lateral spikelet appendages (Lr, lr) with factor pairs in known linkage groups were also a part of this investigation. There is an indication that this factor pair is linked with the one for green versus orange seedling color (Or, or) in group I, with a recombination value of  $38.58 \pm 1.20\%$  obtained from  $F_3$  data.

## SUMMARY

The variety previously classified as *H. intermedium nudihaxtoni* was found to be a 6-rowed barley which differed from ordinary 6-rowed varieties by a factor for the reduction of lateral spikelet appendages, i.e., awns in this case. It is fully fertile.

The normal versus reduced lateral spikelet appendage (Lr, lr) factor pair is inherited as a single-factor difference. Normal was dominant over reduced appendages (hoods or awns) in the crosses studied. Normal appendages on the lateral spikelets occur only in the presence of the factor for 6-row (vv).

The interrelationship of this factor pair with that for green vs. orange seedlings (Or, or) located in group I indicates a linkage with a recombination value of  $38.58 \pm 1.20\%$  as determined from the  $F_2$  genotypes. The normal vs. reduced (Lr, lr) factor pair was found to be inherited independently of factors known to be located in the other six linkage groups.

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## DEFICIENCY SYMPTOMS AND CHEMICAL COMPOSITION OF LESPEDEZA AS RELATED TO FERTILIZATION<sup>1</sup>

R. E. BLASER, G. M. VOLK, AND W. E. STOKES<sup>2</sup>

THE productivity of pasture grasses on sandy soils of the flat pine lands of peninsular Florida is limited primarily by a lack of nitrogen and to a lesser degree by low levels of phosphorus, calcium, potassium, and other mineral elements (2, 3).<sup>3</sup> Thus, it is desirable to grow legumes, such as lespedeza, in association with grasses to maintain favorable nitrogen levels in the soil.

In Florida, lespedeza has been grown principally on the Orangeburg, Tifton, Norfolk, and similar series of west and north Florida. Only occasional and sparse stands have been observed on the sandy soils of the peninsula. A lespedeza test was established on Leon fine sand, typical of a large portion of the flat pine lands of peninsular Florida, to study varieties, growth, and chemical composition as related to fertilization. Certain mineral-deficiency symptoms which were developed with various fertilizer combinations are given in this paper.

### PLAN OF EXPERIMENT

A low phase of Leon fine sand recently cleared and seeded with carpet grass was selected for the test. No fertilizer had been applied previous to establishing this experiment. The native vegetation was saw palmetto (*Serenoa repens*), wire grass (*Aristida* spp.), gallberry (*Ilex glabra*), and slash pine seedlings (*Pinus palustris*). Four varieties of annual lespedeza without replication were fertilized with each of 16 mixtures. The plots were 6 by 12 feet arranged in factorial design. Fertilizers were weighed separately for each plot and broadcast uniformly by hand in May 1939. Inoculated lespedeza seed were immediately broadcast at the rate of 20 pounds per acre, raked in, and rolled. Because of the late seeding date and consequent competition from carpet grass, partial stands resulted. In March 1940 all plots were refertilized with one-half the initial rate of muriate of potash and reseeded as for 1939.

Lepedeza samples for chemical analysis were harvested in July 1940 when plants were in the early bloom growth stage. Common, Tennessee 76, and Kobe varieties of lespedeza were used for chemical analysis. Averages of single chemical analyses of these three varieties are reported. The Korean variety failed irrespective of fertilizer treatment. Soil samples were taken soon after the collection of plant material for the purpose of determining "available" nutrients.

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<sup>2</sup>Associate Agronomist, Soil Chemist, and Head, Department of Agronomy, respectively. Assistance in statistical procedures by Jeffery Dawson, student assistant, and chemical analysis made by Dean Graham, under the direction of Dr. W. A. Leukel of the Agronomy biochemical laboratory, are gratefully acknowledged.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 228.

## RESULTS AND DISCUSSION

## CHEMICAL COMPOSITION AND DEFICIENCY SYMPTOMS OF LESPEDEZA

Several fertilizer mixtures (combinations of lime, superphosphate, and potash), with their effects on chemical composition and growth of annual lespedeza, are given in Table 1 and Fig. 1, respectively.

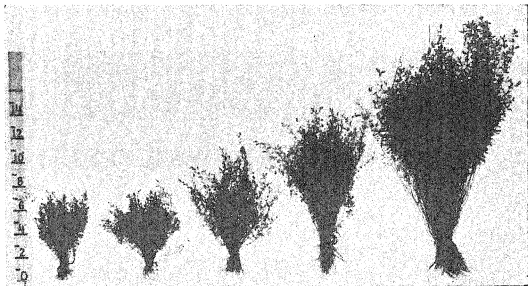


FIG. 1.—Lepsideza plants which received various combinations of nutrients. Left to right: no fertilizer; lime and potash; lime and superphosphate; superphosphate and potash; and lime, superphosphate, and potash.

The chemical composition of lespedeza without fertilization was 0.5714% calcium, 0.1218% phosphorus, 0.3111% potassium, and 1.846% nitrogen. A lime and fertilizer mixture consisting of 1,500 pounds ground limestone, 450 pounds of 18% superphosphate, and 75 pounds of 50% muriate of potash per acre altered the composition as follows: Calcium, 1.0139%; phosphorus, 0.1750%; potassium, 0.3778%; and nitrogen, 2.534%. The differences in chemical composition of fertilized and unfertilized lespedeza are significant for phosphorus and highly significant for calcium and nitrogen.<sup>4</sup>

Similar increases in nutritive elements and protein content of annual lespedezas by fertilization have been reported by others (1, 6, 7, 8).

## PHOSPHORUS

Lepsideza seedlings growing on plots fertilized with lime and potash alone had purple leaf margins, mid-veins, and stems. The leaves were dark green. As growth advanced the leaves and stem branches remained clumped closely to the main stem with leaves in an erect position, as shown in Fig. 2. The leaves later turned to a purplish-green color, typical of phosphorus deficiency in many plants (2, 4, 5).

Phosphorus deficiency symptoms of unfertilized plants differed from those which occurred when lime and potash were applied. The center veins of the leaflets and stems were distinctly purple, with some leaf-

<sup>4</sup>Hereafter in this publication "significant" and "highly significant" indicate that probabilities of .05 and .01, respectively, are taken as levels of significance.

let margins also showing a purple tint. The leaves were yellowish in color and the plants dwarfed.

The characteristic phosphorus deficiency symptoms of lespedeza taken from an unfertilized Leon soil at Gainesville are shown in Fig. 3.

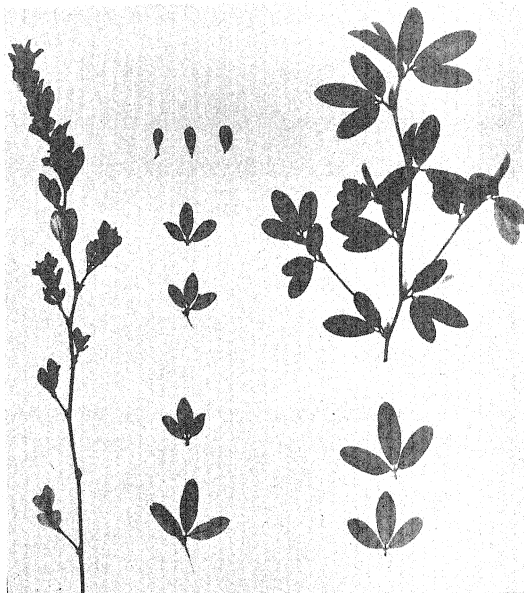


FIG. 2.—Phosphorus-deficient plants and leaves as found when fertilized with a mixture of 1,500 pounds ground limestone and 75 pounds muriate of potash per acre are shown on the left and center of photograph, respectively. Normal growth resulted when 450 pounds of superphosphate per acre was supplied (right). Phosphorus-deficient lespedeza plants which occurred in the presence of potassium and calcium fertilizers on a Leon soil are dwarfed in size with short branching stems. The branching stems and leaves are clumped closely to the main stem in an erect position, as shown on left of photograph. Center shows phosphorus-deficient leaves which are dwarfed in size and vary from a very dull, dark green to a distinct purple and purplish green color. Low center shows a phosphorus-deficient leaf which is purplish in color and possesses dark purple mid-veins and petioles. Lower right shows normal leaves, which are light green in color with white mid-veins. See phosphorus-deficiency symptoms without fertilizer (Fig. 3).

The phosphorus-deficient lespedeza plants were significantly lower in phosphorus content than normal plants which received superphosphate fertilizer (Table 1).

TABLE 1.—*Chemical composition of lespedeza as influenced by lime and fertilizer mixtures.*

Fertilizer applied, lbs. per acre*	Calcium %	Phos- phorus %	Potas- sium %	Nitro- gen %	Ash %
Ca, 1,500; P, 450; K, 75.....	1.0139	0.1750	0.3778	2.534	4.330
Ca, 1,500; K, 75.....	1.0707	0.1153	0.3535	1.968	4.159
Ca, 1,500; P, 450.....	0.8889	0.2000	0.2226	2.380	3.639
P, 450; K, 75.....	0.9280	0.1543	0.2928	2.182	3.560
No fertilizer.....	0.5714	0.1218	0.3111	1.846	2.754
Least significant difference of two means: P=.05	0.1825	0.0461	0.0978	0.309	0.352
P=.01	0.2656	—†	—†	0.450	0.512

\*Fertilizer was applied in 1939. For potash 50 lbs. were applied in 1939 and 25 lbs. in 1940. Ca=ground limestone (93%  $\text{CaCO}_3$ ); P=superphosphate (18%  $\text{P}_2\text{O}_5$ ); K=muriate of potash (50%  $\text{K}_2\text{O}$ ).

†Not significant according to F test.

Stitt (7) planted Korean lespedeza on an unproductive Cecil gravelly loam in 1931 and 1932, and describes seedling growth as follows: "During both seasons the lespedeza plants grew to be about 1 to 2 inches high, became yellowish in color with red leaf margins and stems, after which most of them died." Stitt also reports that Kobe lespedeza averaged 0.083% phosphorus on the same soil when grown without fertilizer, and that phosphorus content and growth were greatly augmented by applying superphosphate to the soil. The mortality of lespedeza seedlings, which Stitt describes, probably was caused by insufficient phosphorus.

#### POTASSIUM.

The potassium content of lespedeza averaged 0.3778% when fertilized with a mixture of 1,500 pounds limestone, 450 pounds superphosphate, and 75 pounds muriate of potash per acre as compared to 0.2226% when potash was omitted. The growth of plants without potash was retarded (Fig. 3). The leaves were mottled and yellowish green in color. Later the leaflets died, starting at the tips, as shown in Fig. 3.

#### CALCIUM

Lepedeza plants fertilized with a mixture of lime, superphosphate and potash were slightly higher in calcium content than lespedeza plants grown with the superphosphate and potash alone. However, both of these treatments increased the calcium content significantly when compared with plants from unfertilized plots. Lime, in combination with other fertilizers, increased the size of lespedeza plants (Fig. 1).

#### NITROGEN

An analysis of covariance shows that the increases in phosphorus content of lespedeza plants and accompanying increase in nitrogen

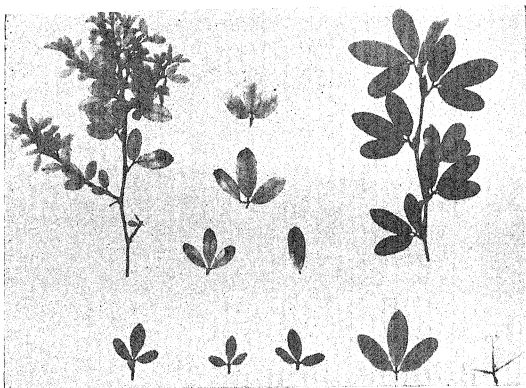


FIG. 3.—Potassium-deficient lespedeza plants and leaves as found when fertilized with a mixture of 1,500 pounds of lime and 450 pounds of superphosphate per acre are shown in the upper left and center. Normal growth resulted when 75 pounds per acre of muriate of potash were supplied (upper right). Potassium-deficient plants were dwarfed in size; leaves were generally mottled and greenish yellow in color, with subsequent burning or browning, starting at tips of leaflets (upper center). Gradual disappearance of chlorophyll and subsequent yellowing typifies incipient potassium deficiency. Lower left shows three leaves of lespedeza taken from plots fertilized with superphosphate and potash. These leaves were thought to be calcium deficient, but analysis did not support this hypothesis. Lower right shows phosphate-deficiency symptoms of a leaf typical of plants without fertilization. This leaf is light yellow in color with purple margins, mid-veins and petioles. A normal leaf is shown at the left of the phosphorus-deficient leaf.

is a highly significant relationship. The regression of nitrogen on phosphorus of five fertilizer treatments and their means is given in Fig. 4.

The increased nitrogen content was not significantly associated with increases of ash, calcium, potassium, or magnesium content of annual lespedeza.

Stitt's data (7) show that the nitrogen content of Korean and Kobe lespedeza was increased by superphosphate and lime applied separately or together on an unproductive soil. Superphosphate alone produced a higher nitrogen content than lime alone for both lespedezas. Weathers (9) found the nitrogen content of lespedeza lower on poor soils than on good soils, but not in direct proportion to phosphorus content of plants. Albrecht and Klemme (1) report that superphosphate did not affect the nitrogen content, but when lime supplemented superphosphate the nitrogen content of Korean lespedeza rose considerably. Apparently, available phosphorus was not

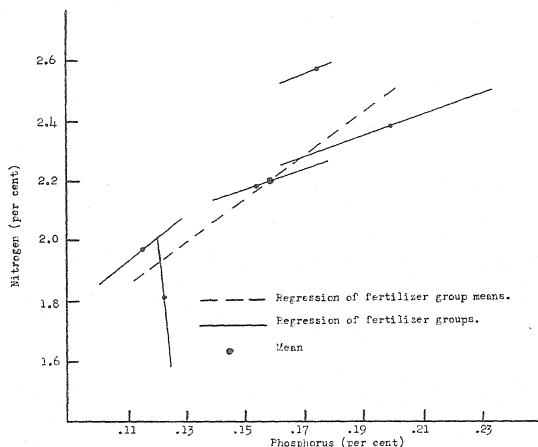


FIG. 4.—Increases in phosphorus content of lespedeza are accompanied by increases in nitrogen as shown by the regression of nitrogen on phosphorus of five fertilizer treatments and their means.

a limiting factor in the soil used for fertility experiments by Albrecht and Klemme because the phosphorus content of Korean lespedeza averaged 0.189% without fertilization. Pieper, *et al.*, (6) found that lime alone increased the nitrogen content, but that phosphorus applied in conjunction with lime was more effective than lime alone.

#### SOIL ANALYSIS

Soil samples were collected from the surface 6 inches on all plots. Organic matter averaged 2.50%. The data on pH, ammonium acetate, exchangeable calcium and potassium, and 0.002 N  $H_2SO_4$  soluble

TABLE 2.—*Soil analysis of Leon fine sand.*

Soil treatment*	pH	Exchangeable calcium, p.p.m.	Exchangeable potassium, p.p.m.	0.002 N $H_2SO_4$ soluble phosphorus, p.p.m.
Ca, 1,500; P, 450; K, 75	5.15	114	8.2	4.8
Ca, 1,500; K, 75	5.14	152	6.0	3.13
Ca, 1,500; P, 450	5.03	173	6.0	4.20
P, 450; K, 75	4.70	95	6.3	3.43
None (virgin soil)	4.73	74	8.7	2.37

\*Refer to Table 1 for symbols.

phosphorus appear in Table 2. Exchangeable calcium, acid-soluble phosphorus, and pH show the effect of calcium and phosphorus amendments. Exchangeable potassium is very low irrespective of fertilizer treatment. This is commonly the case on the lighter sandy soils of Florida except immediately after addition of potassium amendments.

#### SUMMARY AND CONCLUSIONS

A lespedeza test was established on a Leon fine sand, a soil typical of a large portion of the flat pine lands of peninsular Florida. The effect of certain combinations of fertilizer nutrients on mineral-deficiency symptoms, composition, and growth are given.

The calcium, phosphorus, potassium, and nitrogen content of annual lespedeza, sampled during the early bloom stage, was significantly increased by fertilization. Fertilization also increased the plant size.

Phosphorus-deficiency symptoms of lespedeza are described and illustrated. The deficiency symptoms vary depending on the presence of other nutrients. Plant analyses substantiate foliar symptoms of phosphorus deficiency.

Potassium-deficiency symptoms, as found when a fertilizer mixture of lime and superphosphate was used, are described and illustrated. The visible symptoms are corroborated by the chemical composition of the plants.

Phosphorus fertilizer increased the phosphorus content of lespedeza. The increases of phosphorus in lespedeza were accompanied by highly significant increases in nitrogen content.

Soil analysis showed that the virgin Leon soil was low in readily soluble phosphorus, calcium, and potassium.

The elements calcium, phosphorus, and potassium must be added to virgin soils of the Leon series to produce a satisfactory growth of lespedeza.

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## ADAPTATION OF VARIOUS CROPS FOR SUPPLEMENTARY PASTURE<sup>1</sup>

T. E. ODLAND, T. R. COX, AND C. H. MORAN<sup>2</sup>

ONE of the most difficult problems that the Rhode Island dairy farmer has to meet is the providing of sufficient pasturage for his milking herd. Improving permanent pastures by applying commercial fertilizers and lime provides only a partial solution. There are still many weeks during the summer and early fall months when the permanent pasture fails to provide sufficient grazing. The fertilizers applied often build up a surplus when the natural peak in pasture production occurs but still leave a shortage for the remainder of the season.

Many of the most successful dairymen in the state are using a regular system of growing supplementary pasture crops in order to provide additional grazing during the season of short permanent pasture. Rye and vetch, Japanese millet, oats and vetch, sudan grass, and soybeans are among the crops commonly used (3).<sup>3</sup> There are a number of questions often raised as to the best way of growing such crops for pasture purposes. Among these questions are (a) When should each be planted in order to furnish grazing for certain periods? (b) How much feed will be produced and over what period of time? (c) What is the relative value of different crops for grazing purposes?

Little experimental data are available to answer these questions. Most of the experimental work with pastures has been done with permanent types. Woodward and Graves (4) have pointed out that the great variation in carrying capacity of pastures during the season makes it necessary to plan for supplementary pasture crops in order to maintain sufficient grazing. Sudan grass, sweet clover, and soybeans are suggested as possibilities. The authors estimate that a 1,000-pound cow producing 25 pounds of 4% milk will need from 100 to 150 pounds of green grass daily to maintain production. Much depends on the kind and quality of green grass available.

Graves, *et al*, (2) compared the feeding value of pasture grasses when grazed, when fed green, and when fed as hay or silage. Immature grass was found much superior to mature when fed in any of these ways. There was some indication that grass silage was more stimulating to milk flow than grass hay. A system of livestock farming is suggested where most of the land will be in permanent grass and any surplus not used for grazing may be utilized for hay or silage. This surplus may be used either for supplementing the pasture when grazing is short or for feeding during that part of the year when grazing is not available.

Ahlgren, Briggs, and Graber (1) recommend winter rye, oats, sudan grass, and millet, among others, as crops suitable for supplementary

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 237.



pasture in Wisconsin. Suggestions for growing these crops are given. These authors emphasize the necessity of providing such supplementary grazing crops when permanent pastures are short.

In order to secure information on some of these questions, an experiment with various crops for use as supplementary pasture was begun at the Rhode Island Experiment Station in 1938. Results obtained in this experiment will be reported briefly in this paper.

#### DESCRIPTION OF EXPERIMENT

The crops grown included winter rye, winter wheat, spring oats, Japanese millet, and sudan grass. These crops were planted on various dates and harvested when they had reached a good grazing stage. The yields were obtained by weighing the harvested material as cut. Samples were taken for dry matter determinations and for chemical analyses. The various treatments and crops were in either triplicate or quadruplicate plots, each 4×26 feet. The soil is classified as Bridgehampton very fine sandy loam, is underlain by gravel, and is well drained.

The fertilizer application has consisted of 600 pounds per acre of a 5-10-5 grade annually for all crops. No manure has been used. The soil had been well limed before beginning this experiment, the pH being approximately 6.0.

The dates of planting the various crops were as follows: Spring oats, April 15 and 25, May 5; sudan grass, May 20, June 1 and 15, July 1 and 15; millet, May 20, June 1 and 15, July 1 and 15; winter rye, August 10 and 20, September 1, 10, and 20; and winter wheat, August 10 and 20, September 1, 10, and 20.

The oats, rye, and wheat were seeded at the rate of 100 pounds per acre, sudan grass at 40 pounds, and Japanese millet at 25 pounds. A 1-year comparison of 25- and 40-pound rates of seeding for sudan grass was included.

In one set of plots, sudan grass was cut at four different heights ranging from 1 inch to 4 inches, in order to study the effects on recovery of the plants from simulated heavy grazing as contrasted with moderate grazing.

The different crops were cut by means of a small cutter-bar attachment on a garden tractor. The early planted plots of rye and wheat were usually cut once in the fall and two or three times in the spring. The late-seeded plots were cut in the spring only. Oats were cut two to four times in the spring depending on the season. The same was true of sudan grass and millet.

Chemical analyses<sup>4</sup> were made to determine protein content and other feed constituents. Determinations were made on a number of samples of sudan grass for hydrocyanic acid content.

#### EXPERIMENTAL RESULTS

The average yields of green and dry material per acre obtained over the 4-year period are presented in Table 1.

Rye planted at various dates from August 10 to September 20 was ready for grazing on May 8, as an average. It provided grazing over an average period of 34 days. The August seedlings of rye and wheat did not provide as much grazing in the spring as did the September seedlings. The September 20 seeding was not as satisfactory as the seedlings earlier in September. Usually not enough growth on the September 10 and 20 seedlings was obtained in the fall to warrant harvesting.

<sup>4</sup>The authors are indebted to the Agricultural Chemistry Division of the Rhode Island Experiment Station for chemical analyses.

TABLE 1.—*Grazing periods and yields of supplementary pasture crops, 1938-41.*

Date planted	Cutting dates, av.		Grazing period, days*	Yields, tons per acre	
	First	Last		Green	Dry
Rye					
Aug. 10.....	May 8	June 4	34	1.82	0.36
Aug. 20.....	May 8	June 4	34	1.86	0.37
Sept. 1.....	May 8	June 4	34	4.13	0.87
Sept. 10.....	May 8	June 4	34	3.98	0.85
Sept. 20.....	May 8	June 4	34	3.98	0.84
Wheat					
Aug. 10.....	May 8	June 4	34	1.46	0.36
Aug. 20.....	May 8	June 4	34	1.78	0.37
Sept. 1.....	May 8	June 4	34	2.98	0.64
Sept. 10.....	May 8	June 4	34	3.44	0.74
Sept. 20.....	May 8	June 4	34	3.00	0.65
Oats					
April 15.....	June 4	June 28	31	5.62	0.85
April 25.....	June 7	July 1	31	5.97	0.92
May 5.....	June 14	July 10	33	5.72	0.78
Sudan Grass					
May 20.....	July 11	Aug. 23	50	6.54	1.16
June 1.....	July 16	Sept. 1	54	7.64	1.34
June 15.....	July 23	Sept. 8	54	6.08	1.05
July 1.....	Aug. 3	Sept. 18	53	5.73	0.86
July 15.....	Aug. 10	Sept. 25	53	3.46	0.68
Millet					
May 20.....	July 11	Aug. 17	44	7.38	1.21
June 1.....	July 16	Sept. 1	54	9.19	1.49
June 15.....	July 23	Sept. 8	54	9.48	1.40
July 1.....	Aug. 3	Sept. 18	53	7.91	1.28
July 15.....	Aug. 10	Sept. 25	53	4.75	0.80
Rye					
Aug. 10.....	Sept. 29	Oct. 20	29	1.98	0.19
Aug. 20.....	Oct. 2	Oct. 20	25	2.69	0.31
Sept. 1.....	Oct. 20	Oct. 20	7	2.82	0.48
Sept. 10.....	—	—	—	—	—
Sept. 20.....	—	—	—	—	—
Wheat					
Aug. 10.....	Sept. 29	Oct. 20	29	2.40	0.23
Aug. 20.....	Oct. 2	Oct. 20	25	2.71	0.26
Sept. 1.....	Oct. 20	Oct. 20	7	2.05	0.40
Sept. 10.....	—	—	—	—	—
Sept. 20.....	—	—	—	—	—

\*Days between first and last harvests plus 7 days allowance for grazing after last cut.

The average yield of rye over a 34-day period in the spring from the September plantings was 4 tons of green material per acre. Using a figure of 125 pounds per cow per day as pasture requirement

(Woodward), this would furnish enough grazing for two cows over this period.

Winter wheat did not yield as much grazing as the winter rye. It probably could be depended on to produce about three-fourths as much grazing as the rye.

Winter vetch is often seeded with these two crops but was not used in this experiment. General observation has been that, although the yield may not be much more than when the crops are seeded alone, the higher protein content of the feed makes this mixture worth the extra cost of the seed.

Oats planted April 15 to May 5 yielded grazing from June 4 to July 17. The yields averaged 5.75 tons per acre. Usually the period from June 1 to 15 is the flush season on permanent pasture so that the need for supplemental pasture at this time is not as great as later in the season. The May 5 planting was ready for grazing about June 15 and provided an average of 33 days of pasturage. An acre of oats at the rate of yield obtained should produce enough grazing for three cows over this period.

Sudan grass planted from May 20 to July 15 yielded grazing from July 11 to October 1. The May 20 seeding was not as successful on the average as later seedings because weed competition was too great. Seedings made June 1 and 15 and July 1 were the most successful. Sudan grass planted June 1 furnished grazing from July 15 to September 7 with an average yield of 7.64 tons per acre. This is the equivalent of 125 pasture units per acre. Two acres of sudan would produce enough grazing for five cows over a 7-week period. Another seeding made July 1 would lengthen the period to October 1.

Japanese millet planted June 1 provided grazing over a 54-day period from July 16 to September 8. About 9 tons per acre were produced over this period, which was a little more than that of sudan grass. It made a denser growth.

Rye seeded August 10 and 20 furnished grazing during the first 3 weeks of October and the September 1 seeding about a week beginning the middle of October. An average of 2.5 tons of green feed per acre was produced. An acre of rye would furnish enough grazing at this rate to carry two cows over a 3-week period. Winter wheat produced about as much grazing during the fall months as did the winter rye.

Sudan grass cut at different heights showed the best results when cut at a 1- or 2-inch height, indicating that the most material could be expected if some form of rotational grazing were used and the crop grazed closely at each pasturing period. The results are presented in Table 2. When cut to 1-inch height at each harvest, the average yield was 8.45 tons per acre, while the 4-inch cut yielded 6 tons. Sudan grass should be allowed to reach a height of 2 feet or more before grazing to avoid any danger of prussic acid poisoning.

Sudan grass often is reported as a failure due to the poor stands obtained. It was thought that this might be caused by poor seedbed preparation. A comparison was made between loose and firm seedbeds. The results are shown in Table 3.

TABLE 2.—*Comparison of yields of sudan grass planted June 1 and cut at various heights, 1938-41.*

Height of cut, inches	Yield, tons per acre									
	1938		1939		1940		1941		Average	
	Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry
1	7.96	0.86	10.35	1.63	5.14	0.78	10.35	1.94	8.45	1.30
2	6.45	0.73	8.96	1.44	3.99	0.61	9.46	1.77	7.22	1.14
3	5.76	0.62	9.06	1.48	3.54	0.54	8.29	1.59	6.66	1.06
4	4.26	0.49	9.04	1.52	3.06	0.46	7.60	1.44	5.99	0.98

TABLE 3.—*Comparison of yields of sudan grass planted on firm and loose seedbeds, 1938-41.*

Seed-bed preparation	Date of planting	Yield, tons per acre									
		1938		1939		1940		1941		Average	
		Green	Dry	Green	Dry	Green	Dry	Green	Dry	Green	Dry
Firm...	May 20	10.17	1.72	9.11	1.66	—	—	6.89	1.24	6.54	1.16
Loose...	May 20	10.18	1.64	8.31	1.53	—	—	7.09	1.27	6.39	1.11
Firm...	June 1	11.72	1.83	10.70	1.94	1.29	0.22	6.84	1.38	7.64	1.34
Loose...	June 1	9.41	1.47	9.65	1.77	1.18	0.20	6.11	1.28	6.59	1.18
Firm...	June 15	8.94	1.43	9.33	1.70	2.26	0.30	3.79	0.77	6.08	1.05
Loose...	June 15	7.86	1.33	8.61	1.61	1.66	0.22	4.34	0.96	5.62	1.03
Firm...	July 1	8.25	1.24	7.49	1.10	1.24	0.19	5.92	0.89	5.73	0.86
Loose...	July 1	3.94	0.63	3.51	0.53	1.00	0.16	4.98	0.90	3.36	0.56
Firm...	July 15	5.15	0.76	4.42	0.94	1.20	0.24	3.07	0.78	3.46	0.68
Loose...	July 15	2.85	0.43	2.37	0.45	0.71	0.14	2.98	0.77	2.23	0.45

The results show that the firm seedbed usually produced considerably better results. The stand was better, there were less weeds, and the yields were higher. The yields from the loose seedbed were nearly as great as those from the firm bed in many cases, but the weight was represented by a larger percentage of weeds. This was especially true of the late summer plantings which correspond more nearly with the dates of planting in common farm practice in this region.

A 1-year trial of two rates of seeding sudan grass 25 and 40 pounds per acre indicated that another cause of failure may be too light seeding. Considerably higher yields and thicker stands were obtained with the 40-pound seeding rate.

## CHEMICAL ANALYSES

The chemical composition of the different crops varied considerably, depending largely on stage of growth when harvested. The protein content was highest at the earliest harvests. In order to

TABLE 4.—Average composition of supplementary pasture crops.

Date planted	Actual		Oven-dry basis				
	Moisture, %	Protein, %	Protein, %	Fat, %	Crude fiber, %	Ash, %	N-free extract, %
Rye (Spring Growth)							
Aug. 10. ....	74.7	3.46	13.69	3.76	19.47	5.64	57.44
Aug. 20. ....							
Sept. 1. ....	78.8	4.68	22.13	3.06	23.67	10.42	40.72
Sept. 10. ....	83.5	3.55	21.50	3.17	23.38	12.82	39.13
Sept. 20. ....	79.8	4.64	22.38	3.42	24.27	8.83	41.10
Wheat (Spring Growth)							
Aug. 10. ....	76.7	3.13	13.44	3.35	22.43	6.22	54.56
Aug. 20. ....							
Sept. 1. ....	83.6	3.23	19.69	3.39	24.28	8.52	44.12
Sept. 10. ....	84.2	3.66	23.13	3.01	25.60	11.58	36.68
Sept. 20. ....	79.8	4.39	21.75	3.68	26.02	7.84	40.71
Oats							
Apr. 15. ....	85.9	3.45	24.23	4.12	19.80	17.02	34.82
Apr. 25. ....	84.7	3.24	22.57	3.65	21.66	15.97	36.16
May 5. ....	86.3	3.19	23.61	3.88	21.16	15.06	36.28
Millet							
May 20. ....	79.4	2.17	11.15	2.41	28.69	10.65	47.08
June 1. ....	77.7	2.30	11.09	2.34	27.96	11.00	47.59
June 15. ....	78.1	2.74	13.30	2.27	25.99	10.60	47.84
July 1. ....	76.2	3.11	13.19	2.51	26.08	12.46	45.75
July 15. ....	81.9	3.39	18.81	3.15	22.03	11.37	44.64
Sudan*							
May 20. ....	80.7	2.46	13.00	2.60	28.24	9.07	47.07
June 1. ....	79.2	2.33	11.88	2.54	28.68	9.37	47.52
June 15. ....	79.7	3.02	15.69	3.14	23.96	8.36	48.84
July 1. ....	80.1	3.09	16.31	2.77	25.61	8.44	46.87
July 15. ....	72.5	4.73	18.06	2.91	24.45	8.68	45.88
Rye (Fall Growth)							
Aug. 10. ....	80.2	5.19	26.19	4.56	17.07	17.31	34.87
Aug. 20. ....	80.7	4.89	27.56	5.35	15.56	12.13	39.40
Sept. 1. ....	82.3	4.73	26.75	5.26	13.73	11.17	43.09
Wheat (Fall Growth)							
Aug. 10. ....	80.9	5.63	29.56	5.18	19.76	10.13	35.37
Aug. 20. ....	81.2	5.38	28.63	5.98	17.25	15.06	33.08
Sept. 1. ....	79.5	6.05	29.50	4.87	14.74	10.68	40.21

\*HCN content was determined on a number of sudan grass samples from various cuttings. The HCN content in mgm per 100 grams dry weight varied from 1.91 to 4.10 with an average of 3.31.

get a general figure for use in these comparisons, all analyses for any crop planted at a certain date were averaged. The results are presented in Table 4.

The moisture content of the crops ranged from an average of 76% for Japanese millet to 86% for early cut oats. The average of all crops for all dates of planting would perhaps be about 80% as harvested.

The protein content of the crops as harvested varied from 2.17% for Japanese millet planted May 20 to 6.05% for wheat planted September 1 and harvested that fall. The high protein content of the wheat is due to its being harvested at a very early stage in the fall. When harvested in the spring, after more growth had been made, the protein averaged 3.5%. Millet and sudan grass averaged somewhat lower in protein than the cereals. On the oven-dry basis the protein content ranged from 11% in millet to 30% in the fall growth of winter wheat.

The fat content on a dry matter basis ranged from 2.3% in millet to 6.0% in fall-grazed winter wheat. The general average was a little less in millet and sudan grass than in the cereals.

Crude fiber varied from 15% in winter wheat and rye for the fall harvests to 29% in millet and sudan. The relatively high fiber content in millet and sudan reflects the more mature stage that these crops had reached before harvest as compared with early harvested rye and wheat.

The ash content ranged from 6 to 17%. It showed the highest average in oats and the lowest in sudan grass. Millet was intermediate in this respect.

In nitrogen-free extract the range was from 33% on a dry matter basis in winter wheat to an extreme of 57% in winter rye harvested as pasturage in the spring.

Determinations of hydrocyanic acid in sudan grass samples showed that in no case was there enough of this present to be considered toxic to grazing animals. If the content is below 50 mgm per 100 grams of dry matter no ill effects may be expected. In no case was the amount found more than a fraction of this quantity.

Taken as a whole, these analyses indicate that all these crops were satisfactory for pasture purposes on the basis of chemical composition. Kentucky bluegrass, a chief constituent in permanent pastures in this region, usually shows a chemical composition in the early stages of growth of approximately the average of all these crops taken as a whole.

#### DISCUSSION

The experiment points the way to a system of supplementary pasture management that will help provide grazing during periods of the season when permanent pastures are short. Rye planted the first week in September will furnish grazing in the fall and spring. Oats planted during the first week in May will be ready for grazing about the middle of June when permanent pastures usually begin to decline. Two or three plantings of sudan grass and millet from June 1 to July 1 will fill the gap from mid-July to October.

Although yields are shown it is difficult to plan on very definite amounts of pasturage. In favorable seasons more may be provided than necessary. In other seasons the estimates will be too high. Planning to preserve surpluses as silage offers the ideal solution. All of these crops make good silage. If a larger acreage than probably will be needed is provided, the surplus can be utilized for silage. In some years silage made from the early crops may be very useful in helping to overcome a pasture shortage later in the season.

In addition to the supplementary pasture crops included in this experiment, other crops are often available for pasturage. Second crops of mixed hay are often available if necessity requires this for pasturage. Ladino clover planted alone or in mixtures has proved a valuable crop for supplementary pasture purposes in Rhode Island.

The accompanying graph (Fig. 1) shows the normal permanent pasture production curve and indicates means of supplementing this in order to provide sufficient pasturage.

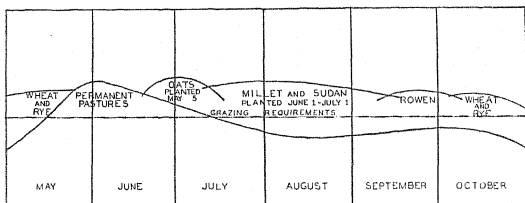


FIG. 1.—Graph of supplementary pasture program.

#### SUMMARY AND CONCLUSIONS

Winter rye, winter wheat, spring oats, sudan grass, and Japanese millet were grown in an experiment to determine their value as crops for supplementary pasture. Various dates of seeding were used. All were found suitable for the purpose.

Winter rye produced more spring pasturage than winter wheat.

Considering both fall and spring grazing, rye and wheat seeded September 1 and 10 were more satisfactory than either earlier or later plantings.

Oats planted May 5 produced grazing at a time when most needed. With earlier plantings the grazing stage coincided more nearly with a time when permanent pastures are at a maximum.

Millet and sudan grass seeded from June 1 to July 1 produced grazing at a critical part of the season. Earlier plantings, especially of sudan grass, were less successful.

No toxic amounts of hydrocyanic acid were found in any of the sudan grass samples.

Chemical analyses showed all materials to be of satisfactory feeding value.

A system of supplementary pasture management is suggested.

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## A STUDY OF THE INCLINED POINT QUADRAT METHOD OF BOTANICAL ANALYSIS OF PASTURE MIXTURES<sup>1</sup>

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A RELIABLE objective method of determining the botanical composition of pasture mixtures would be of great value, particularly if the data obtained would indicate rather accurately the part that each component produced of the total yield of dry matter.

Hanson (2)<sup>3</sup> compared results obtained from the vertical point quadrat apparatus described by Levy and Madden (4) with dry weights of each component from hand separations in studying the native prairie vegetation in the western part of North Dakota. In a comparison made in 1933, *Bouteloua gracilis* and *Carex* spp. made up 65.3% of the total vegetation from the point quadrat readings and only 49.0% as determined from the dry weights of the different components. *Agropyron smithii*, on the other hand, made up only 15.0% of the total as determined from the readings from the point quadrat and 28.3% from dry weights. The differences in the results obtained by the two methods were attributed by the author to the much larger surface of leaf and stem for *Bouteloua* and *Carex* spp. than for *Agropyron* for a given amount of dry weight. Two additional comparisons were made in 1932 on high quality range vegetation. On one of the areas, *Bouteloua* and *Carex* spp. made up 70.0% and 23.6%, respectively, as determined from the point quadrat readings and 66.5% and 30.8% from dry weights of hand-separated material. On the other area *Bouteloua* and *Carex* spp. and *Agropyron* made up 61.1% and 17.4% from the readings as compared to 69.8% and 22.7% from the dry weights. In these two trials the point quadrat readings indicated fairly satisfactorily the percentages of the components in the mixtures as determined from dry weights.

The results obtained from the use of the inclined point quadrat apparatus by Tinney, Aamodt, and Ahlgren (5) indicated that this method was of considerable promise. Hence, it was decided to compare the botanical composition of a number of permanent pasture mixtures as determined by the use of the inclined point quadrat apparatus and from dry weights of each component separated out by hand.

### PROCEDURE

In the spring of 1940 two mixtures were used to obtain data by the inclined quadrat and the list weight methods. One mixture, 4D, consisted mainly of alfalfa, brome grass, and Kentucky bluegrass. The other, 6D, was mainly alfalfa,

<sup>1</sup>Contribution of the Division of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minn. Paper No. 1937 of the Journal Series Minnesota Agricultural Experiment Station. Assistance in the preparation of these materials was furnished by the personnel of the Works Projects Administration, Official Project No. 165-1-71-124, Subproject No. 487. Sponsor: University of Minnesota. 1941. Received for publication November 17, 1941.

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 247.

crested wheatgrass, and Kentucky bluegrass. They were two of six mixtures planted on field D in the spring of 1938 in four randomized blocks. A crop of hay was removed in August, 1938, and the fields were grazed in 1939. The determinations were made in May when the growth was between 6 and 8 inches tall before grazing was started. Four quadrat readings were taken at random on each plot making a total of 16 readings for each of the mixtures. As each of the 10 needles of the quadrat apparatus was pushed down through the vegetation, the species' name for each hit was recorded. Immediately upon the completion of each reading, the vegetation from an area 10×19 inches through which the needles passed in making each quadrat reading was harvested and hand separation made in the laboratory. Dry weights were obtained for each of the components.

In 1941, the two methods were again compared, using a total of 18 mixtures. Six of these were the field D mixtures, two of which were used for the 1940 determinations. The 12 other mixtures were grown on field W in only two randomized blocks and six readings and samples were obtained per plot or 12 per mixture. The 12 mixtures on field W were made up of the same species as those on field D but in somewhat different combinations and rates of seeding. The time of sowing and subsequent management were the same for the mixtures on both fields. The determinations were made during the first half of May when the growth was 12 inches high.

Due to a lack of adequately trained help for separating the Kentucky bluegrass and the crested wheatgrass rapidly and accurately where both occurred in the same mixtures, dry weight percentages were determined of the two together. For these mixtures, the percentages of the two species determined from the point quadrat readings were added together.

### EXPERIMENTAL RESULTS

The composition of each of the two mixtures as determined by the different methods in 1940 is given in Table 1.

TABLE 1.—*Percentage composition of two mixtures determined from dry weights and from point quadrat readings.*

Mixture No.	Source of data for determination	Percentage composition			
		Alfalfa	Brome grass	Kentucky bluegrass and crested wheatgrass	Kentucky bluegrass
4D	Dry weights	51.5	28.9	—	19.6
	Quadrat readings	37.1	17.3	—	45.6
6D	Dry weights	43.7	—	56.3	—
	Quadrat readings	31.9	—	68.1	—

For mixture 4D, the percentage composition of Kentucky bluegrass alone as determined from the point quadrat readings was 45.6 as compared with only 19.6 from dry weights. In mixture 6D, 22.6% of crested wheat grass was indicated from the point quadrat readings. The Kentucky bluegrass and crested wheatgrass were not separated for dry weight determinations. Of these two narrow-leaved species

taken together, the point quadrat readings indicated a higher percentage than was shown by the dry weights.

In contrast, brome grass in mixture 4D, a broader leaved species than either Kentucky bluegrass or crested wheatgrass, was given a lower percentage by the point quadrat than by the dry weight method. Alfalfa, a broad-leaved species, had a lower percentage in each of the two mixtures by the point quadrat than by the dry weight method.

In Table 2, the dry weights in grams per point quadrat hit are given for each mixture for which the percentages were given in Table 1.

TABLE 2.—Grams dry weight per hit as determined from a total of 16 point quadrat readings per mixture.

Mixture No.	Species	Weight, grams	N
4D	Alfalfa	$0.67 \pm 0.05$	16
	Brome grass	$0.53 \pm 0.07$	15
	Kentucky bluegrass	$0.32 \pm 0.03$	16
6D	Alfalfa	$0.76 \pm 0.11$	16
	Kentucky bluegrass and crested wheatgrass	$0.39 \pm 0.03$	16

The difference in grams dry weight per hit between any two constituents in mixture 4D or between alfalfa and the combination of Kentucky bluegrass and crested wheatgrass in mixture 6D were found to be highly significant when subjected to Fischer's "t" test (1).

For mixture 4D, each alfalfa hit by a point quadrat needle indicated over twice as much dry weight in grams as was represented by a Kentucky bluegrass hit. Similarly, each brome grass hit indicated a considerably higher percentage of dry weight than was shown by a Kentucky bluegrass hit. The dry matter per hit indicated for alfalfa in mixture 6D was 0.76 gram compared with only 0.39 gram for the Kentucky bluegrass-crested wheatgrass composite.

The 1941 percentage determinations by the two methods made on 18 mixtures are shown in Table 3.

The results from the determinations on Kentucky bluegrass, Kentucky bluegrass and crested wheatgrass together, and alfalfa in the larger number of mixtures tended to confirm the results obtained from the two mixtures, 4D and 6D, used for the comparisons in 1940.

In 1941, crested wheatgrass was a major constituent only in mixture 6D, where it made up 41.8% of the total as determined from the quadrat readings. This species made up only 3.2% in mixture 5W, 2.9% in 6W, 4.1% in 7W, and 0.4% in 8W as determined from the quadrat readings. Therefore, for the majority of the mixtures, the comparisons in the Kentucky bluegrass-crested wheatgrass column of Table 3 were for Kentucky bluegrass only.

For a number of these mixtures the percentages of Kentucky bluegrass determined from the point quadrat readings were from one and one-half to over two times as great as those determined from the dry

weights. For only two mixtures, 1D and 9W, were the percentages determined by the two methods reasonably close. The average percentages for the 18 mixtures were 57.0 from the quadrat readings and 40.3 from the dry weights. Apparently, the quadrat method overemphasized Kentucky bluegrass and crested wheatgrass by 16.7%.

For the 14 mixtures in which alfalfa was an important constituent,

TABLE 3.—Percentage composition of 18 mixtures as determined from dry weights from hand separations of the vegetation underneath the point quadrat apparatus and from the point quadrat readings, 1941.

Mixture No.	Source of data for determination	Percentage composition				
		Alfalfa	Red clover	Timothy	Brome grass	Kentucky bluegrass and crested wheatgrass
1D	Dry weights	—	5.3	41.2	0.5	53.0
	Quadrat readings	—	2.2	42.2	—	55.6
2D	Dry weights	9.3	0.2	—	60.7	29.8
	Quadrat readings	—	—	—	55.8	44.2
3D	Dry weights	76.0	—	2.0	0.5	21.5
	Quadrat readings	47.2	—	2.5	0.1	50.2
4D	Dry weights	60.5	—	—	21.7	17.8
	Quadrat readings	39.9	—	—	22.8	37.3
5D	Dry weights	67.2	—	—	15.9	16.9
	Quadrat readings	45.3	—	—	22.0	32.7
6D	Dry weights	53.5	—	—	—	46.5
	Quadrat readings	35.3	0.1	—	—	64.6
1W	Dry weights	22.6	—	7.2	38.3	31.9
	Quadrat readings	14.5	—	4.5	35.2	45.8
2W	Dry weights	27.2	—	—	31.3	41.5
	Quadrat readings	13.9	—	—	27.4	58.7
3W	Dry weights	26.5	—	—	32.2	41.3
	Quadrat readings	13.7	—	—	29.9	56.4
4W	Dry weights	35.6	—	—	28.4	36.0
	Quadrat readings	14.6	—	—	29.5	55.9
5W	Dry weights	46.5	—	5.4	—	48.1
	Quadrat readings	24.3	—	6.9	—	68.8
6W	Dry weights	35.1	—	1.7	—	63.2
	Quadrat readings	13.1	—	0.1	0.2	86.6
7W	Dry weights	33.6	—	—	12.2	54.2
	Quadrat readings	17.8	—	—	0.9	81.3
8W	Dry weights	36.9	—	—	2.1	61.0
	Quadrat readings	18.7	—	0.7	—	80.6

TABLE 3.—*Concluded.*

Mixture No.	Source of data for determination	Percentage composition				
		Alfalfa	Red clover	Timothy	Brome grass	Kentucky bluegrass and crested wheatgrass
9W	Dry weights	—	—	12.7	52.1	35.2
	Quadrat readings	—	—	10.0	49.1	40.9
10W	Dry weights	1.3	—	—	54.5	44.2
	Quadrat readings	—	—	—	43.8	56.2
11W	Dry weights	17.7	—	3.6	43.0	35.7
	Quadrat readings	9.4	—	5.3	38.3	47.0
12W	Dry weights	21.1	0.3	—	30.4	48.2
	Quadrat readings	12.8	—	—	24.5	62.7
Averages	Dry weights	40.0*	—	—	29.1†	40.3‡
	Quadrat readings	22.9*	—	—	34.4†	57.0‡

\*Average for 14 mixtures.

†Average for 11 mixtures.

‡Average for all mixtures.

the point quadrat readings indicated consistently much lower percentages than were determined from the dry weights. The average percentage of alfalfa indicated by the readings was 22.9 compared with 40.0 as determined from the dry weights. The percentage of alfalfa was underestimated 17.1% by the point quadrat method. With the exceptions of the percentages of brome grass in mixtures 7W and 10W, the proportions of this species as determined by the two methods were not far different. Why the results for these two should be so different from the others is not clear. For 11 of the mixtures in which brome grass formed an important constituent, the average percentages were 34.4 from the quadrat readings and 29.1 from the dry weights. This shows a tendency for the determinations of the percentages of brome grass from the quadrat readings to be slightly above those for the dry weights.

Timothy was a major constituent of mixture 1D. For this mixture, and for the other mixtures where timothy was a minor constituent, the percentages determined by the two methods do not differ greatly.

In Table 4 are shown the grams of the dry weight per hit for each species from a total of 240 complete point quadrat readings.

TABLE 4.—*Grams dry weight per hit determined from 240 complete quadrat readings on the 18 mixtures.*

Species	Grams dry weight per hit	N
Alfalfa.....	0.67±0.13	36
Timothy.....	0.32±0.22	11
Brome grass.....	0.36±0.09	27
Kentucky bluegrass plus crested wheatgrass..	0.24±0.05	50

The difference in grams dry weight per hit between any two constituents, except between timothy and brome grass, was found to be highly significant when subjected to Fischer's "t" test (1).

Each needle hit of alfalfa represented slightly less than twice as much dry weight as each hit of brome grass or timothy and nearly three times as much as each hit of Kentucky bluegrass and crested wheatgrass taken together.

The results obtained in both years show that the percentages in the mixtures determined from quadrat readings were consistently too low for alfalfa and, with a few exceptions, too high for Kentucky bluegrass alone or for Kentucky bluegrass and crested wheatgrass combined.

These results suggest the possibility that the average increase from April 13 to October 31, 1938, of 18.9% in Kentucky bluegrass for the mixtures in which it was a major component reported by Henson and Hein (3) might be due to overemphasis of this species from the readings of the point quadrat apparatus made on the latter date as compared with the determinations by the methods used previously.

The fairly consistent higher percentages of Kentucky bluegrass and of Kentucky bluegrass and crested wheatgrass combined and the consistently lower percentages of alfalfa derived from the quadrat readings as compared to those from the dry weights suggested that the dry weights in grams per hit for the different species and species combinations as given in Table 4 might be used as correction factors.

Taking the 0.36 gram dry weight per hit of brome grass as the base or 1 and dividing the grams dry weight per hit for each of the others by this figure, the following correction factors were derived: Timothy, 0.9; Kentucky bluegrass and crested wheatgrass, 0.7; and alfalfa, 1.8. Since there were only a few determinations on red clover, a factor of 1.0 was assumed for this species.

Using the simplest method of making the corrections, the alfalfa hits from the point quadrat readings were multiplied by the factor, 1.8; those for timothy by 0.9; those for brome grass and red clover by 1.0; and those for Kentucky bluegrass and crested wheatgrass combined by the factor 0.7, before converting them to percentages.

This method of applying the correction factors to the hits is illustrated in Table 5.

The percentages of each component of the mixture resulting from the corrected hits are as given in Table 6.

TABLE 5.—*Original and corrected hits for mixture 2W of Table 4.*

Constituent	Original hits from a total of 11 readings		Correction factor		Corrected hits
Alfalfa.....	78	×	1.8	=	140
Brome grass.....	153	×	1.0	=	153
Kentucky bluegrass.....	328	×	0.7	=	230
Totals.....	559				523

TABLE 6.—*Percentage composition of mixture 2W as computed from the original hits, the corrected hits, and the dry weights.*

Source of computation	Alfalfa	Brome grass	Kentucky bluegrass
Original hits.....	13.9	27.4	58.7
Corrected hits.....	26.7	29.3	44.0
Dry weights.....	27.2	31.3	41.5

The percentages of each component of this mixture as determined from the corrected hits and from the dry weights were fairly close to those determined from the dry weights.

When the quadrat hits are not available, the correction factors may be applied to the percentages of each of the species in mixtures. The method of applying the correction factors to percentages is shown in Table 7, using mixture 2W as given in Table 4.

TABLE 7.—*Method of applying correction factors to percentages derived from original hits.*

Constituent	Percentages determined from original hits	Correction factors	Corrected percentages
Alfalfa.....	(13.9	×	1.8 = 25.0) ÷ 0.935 = 26.7
Brome grass.....	(27.4	×	1.0 = 27.4) ÷ 0.935 = 29.3
Kentucky bluegrass..	(58.7	×	0.7 = 41.1) ÷ 0.935 = 44.0
Totals.....	100.0		93.5 100.0

Each percentage as determined from the original hits was multiplied by its correction factor and proportional adjustments made so that the total equaled 100.

The correction factors were then applied to the Kentucky bluegrass alone, the Kentucky bluegrass and crested wheatgrass together, and the alfalfa hits for each of the mixtures before converting them to percentages. The percentages determined from the dry weights and from the corrected quadrat readings are given in Table 8.

With a few exceptions, the percentages of each species in the different mixtures as determined from the corrected quadrat readings approached closely the percentages determined from dry weights.

## DISCUSSION

Results from point quadrat readings taken on vegetation made up of a number of species have been used by investigators in various ways for different purposes. Where readings are taken by this method exclusively and when the vegetation is at about the same height each time, results obtained from season to season or year to year may be interpreted to indicate trends. However, where mixtures are made up of plants with coarse parts and others with relatively small or

TABLE 8.—Percentage composition of 18 mixtures as determined from dry weights from hand separations of the vegetation underneath the point quadrat apparatus and from corrected quadrat readings, 1941.

Mixture No.	Source of data for determination	Percentage composition				
		Alfalfa	Red clover	Timothy	Brome grass	Kentucky bluegrass and crested wheatgrass
1D	Dry weights	—	5.3	41.2	0.5	53.0
	Corrected quadrat readings	—	2.7	48.1	—	49.2
2D	Dry weights	9.3	0.2	—	60.7	29.8
	Corrected quadrat readings	—	—	—	64.3	35.7
3D	Dry weights	76.0	—	2.0	0.5	21.5
	Corrected quadrat readings	70.6	—	1.8	0.1	27.5
4D	Dry weights	60.5	—	—	21.7	17.8
	Corrected quadrat readings	60.8	—	—	18.3	20.9
5D	Dry weights	67.2	—	—	15.9	16.9
	Corrected quadrat readings	65.7	—	—	16.8	17.5
6D	Dry weights	53.5	—	—	—	46.5
	Corrected quadrat readings	59.7	0.1	—	—	40.2
1W	Dry weights	22.6	—	7.2	38.3	31.9
	Corrected quadrat readings	27.9	—	4.0	35.6	32.5
2W	Dry weights	27.2	—	—	31.3	41.5
	Corrected quadrat readings	27.9	—	—	28.8	43.3
3W	Dry weights	26.5	—	—	32.2	41.3
	Corrected quadrat readings	27.3	—	—	31.3	41.4
4W	Dry weights	35.6	—	—	28.4	36.0
	Corrected quadrat readings	28.8	—	—	30.7	40.5
5W	Dry weights	46.5	—	5.4	—	48.1
	Corrected quadrat readings	46.4	—	6.3	—	47.3
6W	Dry weights	35.1	—	1.7	—	63.2
	Corrected quadrat readings	28.9	—	0.2	0.2	70.7
7W	Dry weights	33.6	—	—	12.2	54.2
	Corrected quadrat readings	36.9	—	—	1.0	62.1
8W	Dry weights	36.9	—	—	2.1	61.0
	Corrected quadrat readings	38.2	—	0.8	—	61.0
9W	Dry weights	—	—	12.7	52.1	35.2
	Corrected quadrat readings	—	—	10.4	56.6	33.0
10W	Dry weights	1.3	—	—	54.5	44.2
	Corrected quadrat readings	—	—	—	52.7	47.3
11W	Dry weights	17.7	—	3.6	43.0	35.7
	Corrected quadrat readings	19.1	—	5.0	40.8	35.1
12W	Dry weights	21.1	0.3	—	30.4	48.2
	Corrected quadrat readings	26.0	—	—	26.5	47.5



fine parts, the true botanical compositions apparently are not obtained from the readings.

Also, as the botanical composition of mixtures change, the trend as determined from quadrat readings will be toward still further over- or underemphasis, depending on whether the species that is increasing has a low or high amount of dry weight per needle hit.

If, for example, quadrat readings on a pasture mixture indicated 75% alfalfa and 25% Kentucky bluegrass one year and a year later showed 25% alfalfa and 75% Kentucky bluegrass, then a comparison of original and corrected percentages would be as follows:

Percentages determined from	Percentage composition			
	First year		Second year	
	Alfalfa	Kentucky bluegrass	Alfalfa	Kentucky bluegrass
Original hits . . . . .	75	25	25	75
Corrected hits . . . . .	89	11	46	54
Difference . . . . .	14	14	21	21

The difference between the percentages of the two species as determined from the original and corrected hits was 14 the first year and 21 the second. As Kentucky bluegrass became more abundant in the mixture, overemphasis of this species and underemphasis of alfalfa became greater.

When fairly close approximations of the amounts of dry matter contributed by each species in mixtures containing coarse- and fine-leaved plants were desired from the point quadrat readings, the use of correction factors was found necessary. Use of correction factors derived from the grams dry weight per hit for each species resulted in percentages for each species which compare favorably with those determined from dry weights. The results from the use of the correction factors appear to indicate that the material from which they were derived was fairly adequate. Further work has been planned to obtain additional data on this method of procedure.

#### SUMMARY

Data have been presented showing that percentages of certain species in pasture mixtures determined from readings from the inclined point quadrat apparatus were over- or underemphasized when compared with determinations from dry weights.

A method of correcting for the overemphasis of Kentucky bluegrass alone, Kentucky bluegrass and crested wheatgrass together, and for the underemphasis of alfalfa from the readings from the point quadrat apparatus in the mixtures studied in 1941 was derived and used for that purpose.

With a few exceptions, the percentages of these species as determined from the corrected readings from the point quadrat apparatus approached rather closely the percentages determined for them from dry weights.

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FIELD STUDIES OF SMUT RESISTANCE IN OATS<sup>1</sup>T. R. STANTON AND H. C. MURPHY<sup>2</sup>

THE two oat smuts, *Ustilago avenae* (Pers.) Jens. and *U. levis* (Kell. and Sw.) Magn., are prevalent in most years throughout the United States and reduce the yield and quality of the crop. The two methods of control are (a) seed treatment with formaldehyde, Ceresan, or other disinfectants, and (b) the growing of resistant varieties.

Following the discovery, about 20 years ago, of high resistance to the smuts in varieties such as Markton, Black Mesdag, and Navarro, extensive hybridization experiments were conducted in an attempt to transfer this resistance to leading commercial types of oats. Many new varieties and selections with high resistance have been developed from such crosses, several of which already are in commercial production. The most promising strains have been grown in uniform nurseries under a wide range of environment where they were tested for resistance to smut, particularly to specialized races of smut that appeared in certain sections.

## SCOPE OF THE EXPERIMENTS

The first uniform oat smut nurseries were grown in 1935 in cooperation with nine agricultural experiment stations. In the 4 years following, data were obtained from 13 to 15 nurseries each year. The location of the experiment stations and the names of the cooperators are given in Table 1. Their assistance in making these investigations possible is hereby gratefully acknowledged.

## MATERIAL AND METHODS

The same list of resistant varieties was grown at all stations in a given year, but the number of varieties in the nurseries varied from 20 to 38 in different years. In addition, six or seven susceptible check varieties were grown each year.

Seed for the uniform tests usually was grown at Aberdeen, Idaho. All seed was hulled by hand, divided into lots, and sent from Washington, D. C., to the different stations. There it was dusted with smut spores collected locally the previous season. Sixty seeds of each variety were planted approximately 3 inches apart in rows 15 feet long, or slightly longer. At harvest, counts were made of the number of smutted and smut-free plants in each row. When smut infection occurred at a particular station in varieties previously resistant, specimens were collected for making physiologic race determinations.

## EXPERIMENTAL DATA

The average smut infection of each of 75 oat varieties grown in the uniform nurseries is shown by years in Table 2. The varieties and

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<sup>2</sup>Senior Agronomist and Pathologist, respectively, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture.

TABLE 1.—Location of agricultural experiment stations at which uniform oat smut nurseries were grown for 2 or more of the 5 years, 1935-1939, and the names of cooperators.

Location	Cooperators
Northeastern States	
Ithaca, N. Y. ....	H. H. Love, W. T. Craig
Southern States	
Arlington, Va. ....	J. W. Taylor, H. A. Rodenhiser
Statesville, N. C. ....	G. K. Middleton
Experiment, Ga. ....	R. P. Bledsoe, S. J. Hadden
Denton, Tex. ....	I. M. Atkins, P. B. Dunkle
North Central States	
LaFayette, Ind. ....	R. M. Caldwell
Madison, Wis. ....	H. L. Shands
Ames, Iowa. ....	H. C. Murphy, L. C. Burnett
Kanawha, Iowa. ....	H. C. Murphy, L. C. Burnett
St. Paul, Minn. ....	M. B. Moore, E. C. Stakman
Dickinson, N. Dak. ....	R. W. Smith
Manhattan, Kans. ....	J. H. Parker, C. L. Lefebvre, D. B. Creager, E. G. Heyne
Lincoln, Nebr. ....	K. S. Quisenberry, T. A. Kieselbach
Western States	
Aberdeen, Idaho. ....	Harland Stevens, John L. Toews
Pullman, Wash. ....	C. S. Holton
Davis, Calif. ....	C. A. Suneson

selections are grouped on the basis of species, time of maturity, color of lemma, and in some cases on varietal type, with the source of smut resistance (resistant parent) indicated. The classification of some of the newer strains is only tentative.

#### CHECK OR CONTROL VARIETIES

Of the check varieties, Red Rustproof (Appler strain) and Black Mesdag showed the least infection. Canadian and Gothland were decidedly the most susceptible at all stations and to nearly all collections. The average percentages of infection for these varieties for the 5 years were 73.86 and 36.92, respectively. This high infection in the tester varieties indicates that at nearly all stations and in all years the inoculation was effective.

Infection in Black Mesdag must be attributed to the presence of the specialized races of *Ustilago levis* that attack Fulghum. The Fulghum variety, however, with a 5-year average infection of 13.39%, showed decidedly greater susceptibility to these races than did Black Mesdag.

#### RESISTANT VARIETIES AND SELECTIONS

Among the 68 varieties and selections listed as having resistance to smut, 23 were free from infection in all tests. Also, the percentage

TABLE 2.—Average smut infection in 68 smut resistant varieties and selections and 7 check varieties of oats grown from hulled, inoculated seed at 9 to 15 experiment stations.

Group, variety, and source of resistance	C. I. No.*	Total station years grown	Average percentage infection in					All tests
			1935 (9)†	1936 (13)	1937 (14)	1938 (15)	1939 (14)	
Smut-resistant Varieties and Selections, <i>Avena sativa</i> L., var. <i>diffusa</i> Vav. (Common Oat)								
Early yellow:								
Markton resistance:								
Markton X Iogold.	3237	56	—	0.00	0.31	0.00	0.00	0.08
Miomark.	3418	29	—	—	—	0.00	0.00	0.00
South Dakota No. 510.	3419	29	—	—	—	0.65	0.00	0.30
Carleton.	2378	36	0.47	0.00	0.28	—	—	0.23
Smut-Resistant Sixty-Day.	2908	36	0.00	0.00	0.23	—	—	0.09
Markton X Sixty-Day.	2372	9	0.00	—	—	—	—	0.00
South Dakota No. 334	2884	36	2.97	0.17	0.00	1.13	—	0.80
State Pride X Markton.	3491	15	—	—	—	—	—	1.13
Victoria resistance:								
Boone.	3305	29	—	—	—	0.00	0.19	0.09
Victoria X Richland.	3309	29	—	—	—	0.00	0.00	0.00
Tama.	3502	14	—	—	—	—	0.19	0.19
Vicland.	3611	14	—	—	—	—	0.00	0.00
Victoria X Richland.	3500	14	—	—	—	—	0.00	0.00
Victoria X Richland.	3541	14	—	—	—	—	0.00	0.00
Victoria X Richland.	3542	14	—	—	—	—	0.00	0.00
Victoria X Richland.	3304	42	—	0.00	0.00	0.00	0.00	0.00
Victoria X Richland.	3306	27	—	0.00	0.00	—	—	0.00
Victoria X Richland.	3311	27	—	0.00	0.00	—	—	0.00
Victoria X Richland.	3312	15	—	—	—	0.00	—	0.00
Early white:								
Burt resistance:								
Trojan.	2491	22	4.87	1.65	—	—	—	2.96

<b>Midseason yellow:</b>									
Resistance from Markton or Black Mesdag:									
Markton.....	2053	65	1.68	0.00	0.00	0.00	0.00	0.00	0.23
Hancock.....	3346	29	—	—	—	—	—	0.19	0.67
Uton.....	3141	29	—	—	—	—	—	0.00	0.00
Markton × Patterson.....	3480	29	—	—	—	—	—	0.57	0.30
Cornell Smut-Resistant No. 6.....	3610	14	—	—	—	—	—	4.11	4.11
Lee × Victoria.....	3609	14	—	—	—	—	—	0.00	0.00
O. A. C. No. 144 × Markton.....	3326	29	—	—	—	—	0.00	—	0.00
O. A. C. No. 144 × Markton.....	3492	15	—	—	—	—	1.35	—	1.10
Banner × Markton.....	3331	14	—	—	—	—	2.34	—	1.35
Cornell Smut-Resistant No. 5.....	3330	29	—	—	—	—	—	0.34	1.67
<b>Midseason yellowish white to white:</b>									
Markton resistance:									
Bannock.....	2592	65	0.21	0.34	0.00	0.18	0.00	0.00	0.14
Markton × Victory.....	2965	56	—	0.82	0.41	0.00	0.00	0.00	0.29
Minton (Markton × Idamine).....	2374	56	—	0.15	0.26	0.00	0.00	0.00	0.10
Marion.....	3247	56	—	0.72	0.26	0.95	3.57	0.00	1.35
Markton × Victory.....	2590	29	—	—	—	0.54	0.00	0.00	0.28
Markton × Victory.....	2606	29	—	—	—	0.00	0.00	0.00	0.00
Markton × Rainbow.....	3350	14	—	—	—	—	—	0.00	0.00
Markton × Victory.....	2952	36	0.27	0.75	0.34	—	—	—	0.47
Markton × Victory.....	2962	9	1.88	—	—	—	—	—	1.88
Markton × Victory.....	2968	27	—	1.40	1.86	—	—	—	1.64
Markton × Rainbow.....	3243	27	—	0.17	0.00	—	—	—	0.81
Markton × Rainbow.....	3300	27	—	0.15	0.00	—	—	—	0.91
Nicol.....	2925	9	11.87	—	—	—	—	—	11.87
<b>Midseason grayish white:</b>									
Bond resistance:									
Logold × Bond.....	3543	14	—	—	—	—	—	0.46	0.46
Morota × Bond.....	3328	14	—	—	7.36	—	—	—	7.36
Morota × Bond.....	3483	15	—	—	—	6.56	—	—	6.56

\*Accession number of the Division of Cereal Crops and Diseases.

†Number of stations at which nurseries were grown.

TABLE 2.—Average smut infection in 68 smut resistant varieties and selections and 7 check varieties of oats grown from hulled, inoculated seed at 9 to 15 experiment stations.

Group, variety, and source of resistance	C. I. No.*	Total station years grown	Average percentage infection in					All tests
			1935 (9)†	1936 (13)	1937 (14)	1938 (15)	1939 (14)	
Smut-resistant Varieties and Selections, <i>Avena sativa</i> L., var. <i>diffusa</i> Vav. (Common Oat)								
Early yellow:								
Markton resistance:								
Markton X Iogold	3237	56	—	0.00	0.31	0.00	0.00	0.08
Miomark	3418	29	—	—	—	0.00	0.00	0.00
South Dakota No. 510	3419	29	—	—	—	0.65	0.00	0.30
Carleton	2378	36	0.47	0.00	0.28	—	—	0.23
Smut-Resistant Sixty-Day	2908	36	0.00	0.00	0.23	—	—	0.09
Markton X Sixty-Day	2372	9	0.00	—	—	—	—	0.00
South Dakota No. 334	2884	36	2.97	0.17	0.00	—	—	0.80
State Pride X Markton	3491	15	—	—	—	1.13	—	1.13
Victoria resistance:								
Boone	3305	29	—	—	—	0.00	0.19	0.09
Victoria X Richland	3309	29	—	—	—	0.00	0.00	0.00
Tama	3502	14	—	—	—	—	0.19	0.19
Vicland	3611	14	—	—	—	—	0.00	0.00
Victoria X Richland	3500	14	—	—	—	—	0.00	0.00
Victoria X Richland	3541	14	—	—	—	—	0.00	0.00
Victoria X Richland	3542	14	—	—	—	—	0.00	0.00
Victoria X Richland	3304	42	—	0.00	0.00	0.00	—	0.00
Victoria X Richland	3306	27	—	0.00	0.00	—	—	0.00
Victoria X Richland	3311	27	—	0.00	0.00	—	—	0.00
Victoria X Richland	3312	15	—	—	—	0.00	—	0.00
Early white:								
Burt resistance:								
Trojan	2491	22	4.87	1.65	—	—	—	2.96

Midseason yellow:									
Resistance from Markton or Black Mesdag:									
Markton.....	2053	65	1.68	0.00	0.00	0.00	0.00	0.00	0.23
Hancock.....	3346	29	—	—	—	1.12	0.19	0.67	—
Uton.....	3141	29	—	—	—	—	—	0.00	0.67
Markton X Patterson.....	3480	29	—	—	—	0.57	0.00	0.00	0.30
Cornell Smut-Resistant No. 6.....	3610	14	—	—	—	—	4.11	4.11	0.30
Lee X Victoria.....	3609	14	—	—	—	—	0.00	0.00	4.11
O. A. C. No. 144 X Markton.....	3326	29	—	—	—	0.00	—	—	0.00
O. A. C. No. 144 X Markton.....	3492	15	—	—	—	1.10	—	—	0.00
Banner X Markton.....	3331	14	—	—	—	1.35	—	—	1.10
Cornell Smut-Resistant No. 5.....	3330	29	—	—	—	2.34	0.34	—	1.35
Midseason yellowish white to white:									
Markton resistance:									
Bannock.....	2592	65	0.21	0.34	0.00	0.18	0.00	0.00	0.14
Markton X Victory.....	2965	56	—	0.82	0.41	0.00	0.00	0.00	0.29
Minton (Markton X Idamine).....	2574	56	—	0.15	0.26	0.00	0.00	0.00	0.10
Marion.....	3247	56	—	0.72	0.26	0.95	3.57	3.57	1.35
Markton X Victory.....	2590	29	—	—	—	0.54	0.00	0.00	0.28
Markton X Victory.....	2606	29	—	—	—	0.60	0.00	0.00	0.00
Markton X Rainbow.....	3350	14	—	—	—	—	0.00	0.00	0.00
Markton X Victory.....	2952	36	0.27	0.75	0.34	—	—	—	0.47
Markton X Victory.....	2962	9	1.88	—	—	—	—	—	1.88
Markton X Victory.....	2968	27	—	1.40	1.86	—	—	—	1.64
Markton X Rainbow.....	3243	27	—	0.17	0.00	—	—	—	0.81
Markton X Rainbow.....	3300	27	—	0.15	0.00	—	—	—	0.91
Nicol.....	2925	9	11.87	—	—	—	—	—	11.87
Midseason grayish white:									
Bond resistance:									
Iogold X Bond.....	3543	14	—	—	—	—	0.46	—	0.46
Morota X Bond.....	3328	14	—	—	—	—	—	—	7.36
Morota X Bond.....	3483	15	—	—	—	7.36	6.56	—	6.56

\*Accession number of the Division of Cereal Crops and Diseases.

†Number of stations at which nurseries were grown.



TABLE 2.—*Concluded.*

Group, variety, and source of resistance	C. I. No.*	Total station years grown	Average percentage infection in					All tests
			1935 (9)†	1936 (13)	1937 (14)	1938 (15)	1939 (14)	
<i>Avena byzantina</i> C. Koch (Red Oat)								
Early red:								
Resistance from Navarro, Richland, Markton, or Victoria:								
Fulgrain (Strain 1).....	3253	56	—	0.32	0.00	0.00	0.38	0.17
Fulton (Fulgrum X Markton).....	3327	43	—	—	1.61	1.77	0.45	1.29
Richland X Fulgrum.....	3486	29	—	—	—	0.00	0.00	0.00
Richland X Fulgrum.....	3487	29	—	—	—	0.15	0.00	0.08
Brunker.....	2054	36	0.00	0.31	0.00	—	—	0.11
Fulgrum X Markton.....	3220	22	3.10	1.17	0.15	—	—	1.96
Fulgrum X Markton.....	3332	14	—	—	0.64	—	—	0.15
Fulgrum X Markton.....	3333	14	—	—	—	—	—	0.64
Midseason yellow:								
Source of resistance not known:								
Navarro.....	966	56	—	0.00	0.00	0.00	0.00	0.00
Midseason grayish red:								
Resistance probably from a strain of Red Algerian:								
Victoria.....	2401	65	0.00	0.00	0.00	0.00	0.00	0.00
Bond.....	2733	65	0.09	0.99	0.52	2.07	1.48	1.12
Early to midseason red:								
Victoria resistance:								
Ranger.....	3417	29	—	—	—	0.00	0.00	0.00
Nortex X Victoria.....	3535	14	—	—	—	—	0.00	0.00
Nortex X Victoria.....	3526	14	—	—	—	—	1.43	1.43
Nortex X Alber.....	3545	14	—	—	—	—	0.00	0.00
Fulgrum X Victoria.....	3529	14	—	—	—	—	0.17	0.17
Fulgrum.....	3531	14	—	—	—	—	0.00	0.00
Kanota X Victoria.....	3484	15	—	—	—	0.74	—	0.74

<i>Avena nuda</i> L. (Large Naked or Hull-less Oat)									
Early:	Markton resistance:								
	Wash. Station No. 2088/2400.....	3209	65	0.00	0.00	0.00	0.00	0.19	0.04
	Wash. Station No. 688/2088.....	3206	9	0.38	—	—	—	—	0.38
	Wash. Station No. 680/2088.....	3207	9	8.56	—	—	—	—	8.56
	Wash. Station No. 5624.....	3208	22	1.71	4.46	—	—	—	3.34
Check or Control Varieties, <i>Avena sativa</i> L. var. <i>diffusa</i> Vav. (Common Oat)									
Early yellow:	Richland.....	787	8	20.89	—	—	—	—	20.89
Early black:	Monarch.....	1876	65	24.29	24.65	16.25	13.81	22.98	19.93
	Black Mesdag.....	1877	65	0.00	7.53	5.04	2.09	1.72	3.44
Midseason white:	Canadian.....	1625	65	65.96	72.51	72.21	81.83	71.06	73.86
	Gothland.....	1898	65	33.44	34.47	34.81	38.17	42.20	36.92
<i>Avena byzantina</i> C. Koch (Red Oat)									
Early red:	Fulghum.....	708	65	7.20	12.48	17.21	16.05	11.55	13.39
Midseason red:	Appler.....	1815	64	0.00	1.33	0.81	0.37	0.21	0.56

\*Accession number of the Division of Cereal Crops and Diseases.  
†Number of stations at which nurseries were grown.

\*Accession number of the Division of Cereal Crops and Diseases.

<sup>†</sup>Number of stations at which nurseries were grown.

of smut infection was exceedingly low in nearly all the varieties and selections tested. Of the 45 remaining varieties, only 18 showed an average infection above 1% for all station years. Of these latter, Nicol showed such a high infection that it should not have been classed as a resistant variety.

Of the original smut-resistant varieties, Markton showed an average infection of 1.68% when grown at nine stations in 1935 but was free from smut in the other years. The presence of infection in Markton in 1935 probably was due to mechanical mixtures or natural hybrids. Navarro and Victoria were entirely free from infection at all stations in all years. Bond showed some smutted panicles at one or more stations in all years, averaging 1.12% infection for 65 station years. The infection in Bond was due to the specialized races that attack strains of the Red Rustproof variety. It is evident that Markton, Navarro, and Victoria are outstanding varieties for use in breeding for smut resistance. These results are fully in accord with those obtained by Reed (5)<sup>3</sup> in his extensive studies on the reaction of varieties to physiologic races of the oat smuts. The susceptibility of Bond to the races attacking Red Rustproof oats makes it less valuable as a source of resistance. Likewise, Black Mesdag, one of the varieties used as a check in these tests, because of its susceptibility to the covered smut races attacking Fulghum, is less desirable as a parent.

The selections from the Victoria×Richland cross (XS1098) were the most resistant as a group. Of the 11 strains of this group tested, only Boone and Tama were slightly infected, and this probably was due to mixtures. The outstanding resistance of selections from this cross already has been reported (13, 14). These selections probably represent the most outstanding available material for use in further breeding for smut resistance. The early yellow strains originating from crosses with Markton also have been outstandingly resistant. The selection C. I. 3237, from the Markton×Iogold cross, and Miomark (S. Dak. No. 40) were highly resistant. Trojan had an average infection of 2.96% for the 22 station years grown and thus cannot be considered as being highly resistant.

The midseason white oat selections as a group were not so consistently resistant as the two groups just mentioned. The two selections derived from the Morota×Bond cross, viz., C. I. Nos. 3328 and 3483, did not show satisfactory resistance. The average infections, 7.36% and 6.56%, respectively, were higher than for the check varieties, Black Mesdag and Appler (Red Rustproof), in the same years.

Uton showed no infection in the uniform tests in the 2 years in which it was grown. It also was resistant to a specialized race of *Ustilago avenae* occurring in Utah (10, 15).

Of the new varieties and selections classified as belonging to the red oat group, *Avena byzantina*, Richland×Fulghum (C. I. 3486), Ranger, Nortex×Victoria (C. I. 3535), Nortex×Alber (C. I. 3545), and Fultex were free from smut in the years tested. The Fulton variety recently distributed in Kansas showed an average infection of 1.29% for 43 station years.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 257.

The hull-less selection (C. I. 3209), from Pullman, Wash., had an average infection of only 0.04% for 65 station years, a remarkable record for a hull-less oat. The other three sister naked selections were discontinued after 1 or 2 years' test because of susceptibility.

The origin and development of many of the varieties and selections listed in Table 2 have been reported previously (1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14). Others regarding which very little has been published are discussed below.

Miomark (S. Dak. No. 40), highly resistant to smut, was produced by Matthew Fowlds, formerly of the South Dakota Agricultural Experiment Station, Brookings, S. Dak., by backcrossing a selection of Iogold $\times$ Markton with the Markton parent. Selection Nos. 334 and 510, which also have been highly resistant to the smuts, were originated at the same station by Fowlds. "Swedish Select $\times$ Kilby Hull-less was the original cross. A naked selection from this cross was backcrossed twice to Richland and finally to the Markton variety."<sup>4</sup>

The two highly resistant selections bearing C. I. Nos. 2372 and 2908 are sister selections of Carleton, the history of which has been published (8). They are similar to Carleton morphologically.

Selection C. I. 3941 was developed by the Wisconsin Agricultural Experiment Station at Madison, Wis., from a cross between State Pride (Wisconsin Pedigree 7) and Markton. The cross was made in 1928 by B. D. Leith and R. G. Shands. Since that time Leith and H. L. Shands have selected it for agronomic qualities and more recently tested it for smut resistance.

The selection C. I. 3480 was developed from a cross between Markton and Patterson made by C. F. Noll of the Pennsylvania Agricultural Experiment Station at State College, Pa. It was slightly susceptible to smut.

The two selections designated as Cornell Smut-Resistant Nos. 5 and 6, C. I. Nos. 3330 and 3610, respectively, were originated by H. H. Love and W. T. Craig at the Cornell University Agricultural Experiment Station, Ithaca, N. Y., in cooperation with the Division of Cereal Crops and Diseases. No. 5 was selected from a cross between a selection from a field of oats in New York and a selection from a Black Mesdag $\times$ Sixty-Day cross. Its resistance was not satisfactory, and it therefore has been discontinued. No. 6 was originated from a cross between Cornell Smut-Resistant No. 5 and Cornellian. Although resistant at Ithaca, No. 6 showed the highest infection of all the selections included in the uniform tests.

The winter oat selection C. I. 3609 was selected by George J. Wilds, President and Head Plant Breeder, Coker's Pedigreed Seed Co., Hartsville, S. C., from a mass population of the Lee $\times$ Victoria cross, furnished by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture. This selection was free from smut infection. However, it has not been named or distributed because sib strains of equally high resistance have proved to be superior in yield and quality.

The selections bearing C. I. Nos. 3326 and 3492 were originated

<sup>4</sup>Letter of October 28, 1938, from Matthew Fowlds to T. R. Stanton.

at the Wisconsin Agricultural Experiment Station, Madison, Wis., from a cross between O. A. C. 144 and Markton made by B. D. Leith. The selection C. I. 3326 was entirely free from smut in the 2 years in which it was tested. The second selection, C. I. 3492, showed some susceptibility.

The selection C. I. 3331 was developed from a cross between Banner and Markton by the Washington Agricultural Experiment Station, Pullman, Wash. It did not prove highly resistant in the 1 year tested.

Among the original selections from the Markton×Victory cross the one bearing C. I. No. 2606, a sib of Bannock and similar to it in smut reaction, has been of some promise because of a very stiff straw. The strains C. I. Nos. 2952, 2962, 2965, and 2968 represent reselections of the Markton×Victory cross, made at the Aberdeen Substation, Aberdeen, Idaho, by T. R. Stanton, F. A. Coffman, and L. L. Davis in 1930. Several of these reselections showed slight susceptibility.

The selection bearing C. I. No. 3300, from the Markton×Rainbow cross, is identical, both pathologically and morphologically, with Marion (9). The selection C. I. 3243 is similar and closely related to Marion (9). Nicol was believed to be resistant to certain races of the oat smuts occurring in Europe. It was introduced through W. Nicoliasen of the University of Halle, Halle-Salle, Germany, in 1931 by the U. S. Dept. of Agriculture. It has proved to be of no pathologic or economic value in this country.

The selection C. I. No. 3543 (35-956), from the Iogold×Bond cross, was originated at Ames, Iowa. The cross was made in 1932 by H. C. Murphy. The two selections C. I. Nos. 3328 and 3483 likewise were developed by Murphy at Ames from a cross made in 1932 between Morota and Bond. These selections did not show altogether satisfactory resistance.

Among the early red oats, the two selections C. I. Nos. 3486 and 3487, from the Richland×Fulghum cross, were developed by the Kansas Agricultural Experiment Station, Manhattan, Kans., in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture. The cross was made by F. A. Coffman in 1928, and these selections were developed primarily under the supervision of J. H. Parker, formerly of the Kansas Agricultural Experiment Station. They have been of special interest because their resistance is derived from Richland, a variety resistant to the specialized races of the oat smuts that are so virulent on Fulghum oats.

The history of the three Fulghum×Markton selections, C. I. Nos. 3220, 3332, and 3333, is similar to that of Fulton (8), a closely related selection. It has not been possible to obtain selections from this cross with complete resistance to the smuts. The instability of Fulghum in morphological characters appears to be paralleled by a similar behavior in its pathological reactions.

The selection C. I. No. 3535 (Tex. 11-35-41) was originated in cooperative experiments at Substation No. 6, Denton, Tex., by I. M. Atkins from the same cross (Nortex×Victoria) from which Ranger

and Rustler (10) were selected. It showed very high resistance to smut. The selection C. I. 3526, from the same cross, was not altogether satisfactory for resistance to smut.

The highly smut-resistant selection C. I. No. 3545 (Tex. M10-11) was developed by P. C. Mangelsdorf and E. S. McFadden in cooperative experiments at College Station, Tex., from a Nortex×Alber cross. The selection C. I. No. 3529 (Tex. 12-34-13) is a full sib of Fultex and has essentially the same history (10). This selection, however, was not so highly resistant to the smuts as was Fultex.

The one selection from the Kanota×Victoria cross, C. I. 3484, showed some susceptibility. It was developed by I. M. Atkins in cooperative experiments at Substation No. 6, Denton, Tex., the cross having been made by F. A. Coffman in 1930.

The four selections of hull-less oats, C. I. Nos. 3206, 3207, 3208, and 3209, and Wash. Nos. 688/2088, 680/2088, 5624, and 2088/2400, respectively, were developed at Pullman, Wash., by E. F. Gaines from a cross between Markton and a naked oat known as "Large Hull-less". The selection C. I. 3209 has been remarkably resistant to the smuts of oats. It is probable that the slight infection occurring in the population in 1939 resulted from a stray plant.

#### SUMMARY

Uniform tests of oats for smut resistance were conducted for 5 years at 9 to 15 stations each year. Local collections of smut were used at each station.

The Canadian and Gothland check varieties showed high infection to nearly all collections of smut, averaging 73.86% and 36.92%, respectively. Comparable smut percentages for the other check varieties, Richland, Monarch, Black Mesdag, Fulghum, and Appler, were 20.89%, 19.93%, 3.44%, 13.39% and 0.56%, respectively.

Navarro and Victoria were entirely free from infection at all stations in all years. Markton showed an average infection of 1.68% in 1935, but this probably resulted from mechanical mixtures as the variety was free from smut in the other 4 years. Bond showed some infection in all years due to its susceptibility to the specialized races that attack the Red Rustproof strains.

Of the 68 varieties and selections considered as having resistance to smut, 23 were free from any infection whatever in all years tested. In general, the amount of infection for most years was very low for nearly all the varieties and strains tested. Only 18 showed an average infection exceeding 1%.

Of the various groups of selections, that from the cross between Victoria and Richland was the most resistant to smut. The Bond crosses were the most susceptible of any group of resistant selections.

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## CROSS FERTILITY RELATIONSHIPS OF "GOLDEN ANNUAL" SWEET CLOVER WITH COMMON SPECIES OF MELILOTUS<sup>1</sup>

I. J. JOHNSON<sup>2</sup>

AN annual type of yellow sweet clover, resembling in general appearance *M. officinalis* and supposed for some time to be an annual form of this clover, was observed by Dr. S. N. Smith in the fall of 1934 at Sioux City, Iowa.

In the late spring of 1934 Dr. Smith had obtained from the Farm Crops Subsection of the Iowa Experiment Station a large number of remnant lots of both biennial white and biennial yellow sweet clover and planted each lot in rows at Sioux City, Iowa. The particular row in which this annual yellow plant was found was planted with seed from a lot of *Melilotus alba* obtained through the U. S. Dept. of Agriculture, Division of Forage Crops and Diseases, from Mongolia, China.

Although this plant made a typical annual growth and bloomed rather freely in 1934, the flowering date was so late it was feared that none of the pods would produce mature seed before frost came. Sixteen rather immature seeds were harvested and turned over to Prof. H. D. Hughes of the Farm Crops Subsection of the Iowa State College for planting and observation. Plants established in pots in the greenhouse during the winter made a good growth and bloomed freely. As summer came on they were transferred out of doors where they were grown in comparison with the annual white sweet clover, Hubam. The general habit of growth and appearance of the two clovers was very similar, except for the color of the flowers and the fact that the annual yellow appeared to be somewhat later in maturity.

In 1936 open-pollinated seed produced by these plants, all of which seemed to be very similar, was planted in individual rows in the field. At the end of the season some rows, and particularly certain plants in these rows, made a somewhat more vigorous growth and appeared to be earlier in maturity than others, though in general the crop was again observed to be remarkably uniform.

Seed of the most vigorous and earliest individual plants was saved, and in the spring of 1937, 11 plant-row seedlings were made. Observations again indicated remarkable uniformity between the different rows allowing little opportunity for selections, but 31 plant-rows were grown in 1938 and 35 in 1939. On the basis of such slight differences in vigor of growth and maturity as could be observed, the number was reduced to four plant-rows in 1940.

In the spring of 1937 the small amount of seed available was divided with Dr. S. N. Smith, who had made the original selection,

<sup>1</sup>Contribution from the Farm Crops Subsection, Iowa Agricultural Experiment Station, Ames, Iowa. Journal paper J-939. Project 152. Received for publication November 22, 1941.

<sup>2</sup>Research Professor.



and this was increased by him and the Michael-Leonard Seed Company. In 1939, seed of this annual type of yellow-flowered sweet clover was placed on the market by the Michael-Leonard Seed Company under the name, Golden Annual. Although this variety had its origin from a single plant, its seed production and general vigor has not been reduced as a result of close breeding.

As no annual strains of yellow-flowered sweet clover other than *Melilotus indica* had been generally known, it seemed desirable to determine definitely by a series of crosses between the different species whether this strain should be classified as *officinalis*. Morphological observations by Dr. R. N. Martin, of the Division of Plant Exploration and Introduction, U. S. Dept. of Agriculture, had indicated the probability that this was an annual form of *M. suaveolens*.

### MATERIAL AND METHODS

In October 1940 seed of Golden Annual, Redfield Yellow (*M. suaveolens*), Hubam, Iowa late white (*M. alba*), and Madrid Yellow (*M. officinalis*) was planted in the greenhouse. Soon after emergence the seedlings were given illumination during the night with 200-watt Mazda lamps and the annual as well as biennial plants made a vigorous growth and flowered in January. Florets were emasculated before anthesis with an air suction provided by a filter pump. To facilitate removal of anthers a glass tip was connected to the air inlet and drawn out to a very fine bore just sufficiently large to permit passage of an anther. Two days after emasculation pollen was collected by tripping with a toothpick the florets of the male parent when at anthesis and the pollen was applied to the stigma of the emasculated florets of the appropriate female parent. In all cases, when possible, the male parent used in the cross possessed a dominant marker. The following inter- and intra-specific crosses were made: *M. alba* × Golden Annual, *M. alba* × *M. suaveolens*, *M. officinalis* × Golden Annual, *M. officinalis* × *M. suaveolens*, *M. suaveolens* × Golden Annual, and *M. alba* × *M. alba*. The cross between two varieties of *M. alba* was made as a check on the completeness of cross pollination with the technics employed in this study and to serve as a basis for comparing pod and seed set in the crosses involving different species.

The success of cross pollination was measured by recording the number of pods set and the number of seeds formed. All plants used in crosses were also self-pollinated to provide a comparison of seed weights from self- and cross-pollination.

The chromosome number of Golden Annual was determined on root tips from seed germinated on blotting paper.

### EXPERIMENTAL RESULTS

From the data given in Table 1 it is evident that Golden Annual should be classified as an annual variety of *M. suaveolens*. The pod and seed set of Golden Annual when crossed to *M. alba* and to *M. officinalis* was comparable to that obtained when Redfield, a variety of *M. suaveolens*, was crossed to these two species. Approximately 50% of the florets of *M. alba* formed pods when pollinated by either Golden Annual or Redfield. Although the number of seeds set was somewhat higher in crosses between *M. alba* and Redfield, this was accounted for in one plant of *M. alba* that as a female parent pro-

duced almost all twin-seeded pods. This plant, unfortunately, was not used in crosses with Golden Annual. Also, when either of the two varieties was crossed with *M. officinalis*, only a few pods with abortive seeds were formed. Finally, in crosses between Redfield and Golden Annual 85% of the florets crossed formed pods, some of which were two-seeded.

TABLE 1.—Number of florets pollinated, pods and seeds set, and the percentage pod set with number of seeds per pod from inter- and intra-specific crosses in *Melilotus*.

Cross	Florets pollinated	Pods Set	Seeds Set	Per cent pod set	Seeds per pod
<i>M. alba</i> × Golden Annual.....	94	45	38	48	0.84
<i>M. alba</i> × <i>M. suaveolens</i> .....	68	35	41	51	1.17
<i>M. officinalis</i> × Golden Annual.....	75	4	0	5	0.00
<i>M. officinalis</i> × <i>M. suaveolens</i> ..	73	5	0	7	0.00
<i>M. suaveolens</i> × Golden Annual.....	62	53	62	85	1.17
<i>M. alba</i> × <i>M. alba</i> .....	31	24	24	78	1.00

The extent of cross pollination between Redfield and Golden Annual compares favorably with that obtained between the two varieties of *M. alba*. In this cross 78% of the florets produced pods all of which were single seeded.

These results indicate, therefore, that Redfield and Golden Annual each is partially cross fertile with *M. alba*, cross sterile with *M. officinalis*, but completely cross fertile between themselves.

The cross fertility relationships of Golden Annual, *M. suaveolens*, and *M. alba* measured by comparing the weight per seed of selfed and crossed seed is shown in Table 2. The average weight of the selfed seed was based on the mean of all female parents used in proportion to the number of crossed seeds obtained on each plant. The results show a very definite decrease in weight of the crossed seed between *M. alba* and either Golden Annual or *M. suaveolens*. The partial cross fertility between these two species of *Melilotus* is therefore not only reflected in a lower percentage of pod and seed set but also in a physiological unbalance resulting in reduced seed size. In contrast, the weight of the crossed seed from *M. suaveolens* × Golden Annual was equal to or slightly higher than the selfed seed of the female parents,

TABLE 2.—Weight in mgm of seed from self pollination in comparison with cross pollination in inter- and intra-specific crosses in *Melilotus*.

Cross	Weight per seed in mgm		Relative weight of crossed seed*
	Selfed seed	Crossed seed	
<i>M. alba</i> × Golden Annual.....	2.51	1.22	49
<i>M. alba</i> × <i>M. suaveolens</i> .....	2.61	1.35	52
<i>M. suaveolens</i> × Golden Annual.....	2.54	2.64	105
<i>M. alba</i> × <i>M. alba</i> .....	2.66	2.71	102

\*Based on the weight of selfed seed as 100.

indicating, as in the cross fertility study, complete compatability between the two varieties. The relative weight of crossed seed in this combination was essentially the same as that obtained in the crosses between the two varieties of *M. alba*. These results further indicate that Golden Annual should be classified as a variety of *M. suaveolens* and demonstrate the possibility in this genus of basing the species classification on a genetic rather than on a morphological basis.

Investigations are in progress to determine the inheritance of the annual character of *M. suaveolens* and to study the genetic relationship between this type and the annual *alba*. Results in the  $F_1$  generation show that the annual *suaveolens* is dominant over the biennial form as is true in crosses between biennial and annual forms in *M. alba*. Since the annual forms are considered as mutants, the occurrence of dominant mutants in each of the two species widely separated in their geographical origin is of unusual interest. It should be emphasized, however, that the more common biennial recessive forms may be the mutant rather than the normal type. Among the 14 species of *Melilotus* described by Martin<sup>3</sup> 6 are annual, 4 are biennial, and 4 have both annual and biennial types. The distribution in numbers of the annual and biennial species therefore is nearly equal. Studies on the homology of the genes for annual growth habit among these species where crosses can be made should give some information on the phylogeny of the species of *Melilotus*.

The chromosome number of Golden Annual as determined by Dr. J. E. Sass, of the Botany Department, Iowa State College, shows 16 in the somatic tissue. This chromosome number is the same as reported for all species of *Melilotus* where counts have been made.

### SUMMARY AND CONCLUSIONS

Golden Annual, a variety of yellow-flowered sweet clover, was obtained from a single yellow-flowered plant in seed introduced by the Division of Forage Crops and Diseases, U. S. Dept. of Agriculture, from Mongolia, China.

From a series of crosses between Golden Annual and varieties of *M. alba*, *M. officinalis*, and *M. suaveolens* the cross-fertility relationship as measured by seed setting and seed weight indicate that this variety is an annual form of *M. suaveolens*.

In the  $F_1$  generation, the annual form is dominant over the biennial in the same species. More detailed data from subsequent generations pertaining to the homology of the genes for annual growth habit in inter-specific crosses will be reported later.

<sup>3</sup>MARTIN, ROBERT F. Preliminary report on systematic studies of *Melilotus*, unpublished data. Div. of Plant Exploration and Introduction, U. S. Dept. of Agriculture.

# CONTOUR TILLAGE OF CORN UNDER NEW JERSEY CONDITIONS IN RELATION TO SOIL AND WATER CONSERVATION, CROP YIELDS, AND VARIOUS SOIL PROPERTIES<sup>1</sup>

H. C. KNOBLAUCH<sup>2</sup>

THE growing of corn for silage presents one of the major soil conservation problems on the sloping lands of the New Jersey dairy region. Observations made in New Jersey indicated a considerable variation in depth of top soil and growth of corn on numerous areas. Some of the observed variations in soil and condition of growth could be correlated with differences in method of tillage or previous soil management. Moderate to steep slopes that had been planted to corn for several years with rows up and down hill were the most seriously affected. Many of these areas have been abandoned because of unsatisfactory crop yields. Other fields with slopes as extreme as the abandoned areas, but under different methods of tillage or management, were observed to have suffered only moderate erosion and to be of average productivity.

In order to obtain information on some of the observed differences, the Soil Conservation Service and the New Jersey Agricultural Experiment Station established a series of runoff measuring plots at the Dairy Research Farm of the New Jersey Agricultural Experiment Station at Sussex, N. J. The objective of the experiment was to determine the effect of contour tillage of corn on soil and water losses, crop yields, and certain soil properties.

## THE EXPERIMENTAL AREA

The field studies were conducted on a soil of the Dutchess series, which, together with closely related series, is representative of approximately 3½ million acres of land in the Northeast and includes the principal soil types found in the northern extension of the Great Valley. The particular area selected was considered representative in soil, slope, and topography of many of the areas used for corn growing in the Northeast.

The soil of the experimental area is designated as Dutchess loam. However, current correlation based upon field inspection may place the soil in the Albion series. Pending the confirmation of the field correlation with laboratory studies, the soil will be designated as Dutchess loam. The soil has developed from the underlying shale and glacial material. Under virgin conditions, there is evidence of podzolic development. When cultivated and not severely eroded, the soil has a brown to grayish brown friable A horizon, 0 to 8 inches in depth.<sup>3</sup> The B horizon is light brown in color and more compact than the A. The degree of compactness

<sup>1</sup>Joint contribution from the Soil Conservation Service, Office of Research, and the New Jersey Agricultural Experiment Station, New Brunswick, N. J. Received for publication November 24, 1941.

<sup>2</sup>Formerly Project Supervisor, Soil Conservation Research, New Brunswick, N. J.; now Senior Soil Technologist, Office of Experiment Stations, U. S. Dept. of Agriculture.

<sup>3</sup>The experimental area was only slightly eroded and thus the surface soil consisted largely of A material.

increases in the subsoil. There is evidence of imperfect drainage at depths of from 24 to 30 inches. The land slope of the experimental area is fairly uniform, averaging 16.5%.

Drainage was diverted above the plots. During the 10 years preceding initiation of the study, the field was managed as a meadow. The mixture plowed under in the fall of 1936 consisted of a heavy sod of bluegrass, orchard grass, timothy, and weeds.

### EXPERIMENTAL INSTALLATION

In the spring of 1937, six plots, 14 feet wide and 70 feet long, were laid out lengthwise of the slope and planted with Mercer County White Cap corn to be harvested for silage. Three of the plots were planted with the slope and three planted on the contour. No other experimental variable was introduced in the study. All plots were treated annually with 300 pounds of 5-10-5 fertilizer and limed as needed to maintain pH of approximately 6.5. Except for the first year, the plots were spring plowed. A 42-inch row spacing and 9-inch hill spacing was used throughout the experiment. During cultivation operations, the rows were hilled so that a ridge with a height of about 4 inches and a base of from 8 to 12 inches was produced. All plots were planted to corn each year from 1937 to 1940, inclusive, with no change in row direction introduced on the plots from the original layout. Data on yields were taken on all plots each year.

Equipment for measuring soil and water losses was installed on four plots, two of each treatment, during the summer and fall of 1937. Instruments for measuring rate of runoff were installed on one plot of each treatment during the summer of 1938.

Daily records of soil-moisture tension were obtained during the growing season through the use of tensiometers (4),<sup>4</sup> with cups located at three different depths in the soil profile under the two treatments.

Soil samples were taken from the A horizon of each plot in the fall of 1937 and again in the fall of 1940 for total carbon and total nitrogen determinations.

## RESULTS AND DISCUSSIONS

### SOIL AND WATER LOSSES

From the standpoint of the fundamental aspects of land use and conservation of soil resources, the comparison of soil and water losses as given in Table 1 is a measure of the desirability of the two methods of planting.

An analysis of variance (5) of the runoff data indicated that one can state with considerable confidence that contour planting of corn under conditions similar to those that existed in this experiment will result in highly significant reductions in soil loss and significant reductions in water loss. The average annual precipitation of 46.21 inches during the period of this study was above the Sussex, N. J., 36-year average of 43.44 inches (6).

In this experiment contour planting was most effective in reducing soil losses during the growing season. The contour ridges usually began breaking on the lower portion of the slope shortly after harvesting the corn crop. During December, January, and February, soil losses from contoured plots may equal the losses from plots tilled with the slope for individual storms.

<sup>4</sup>Figures in parenthesis refer to "Literature Cited", p. 269.

TABLE 1.—*Effect of row direction upon erosion and runoff from silage corn, Soil Conservation Experiment Station, Sussex, N. J.\**

Total precipitation		Cultivated on contour		Cultivated with slope	
Year	Inches	Soil loss per acre, tons	Runoff, inches	Soil loss per acre, tons	Runoff, inches
1937-38.....	49.88	1.17	3.41	10.24	6.65
1938-39.....	47.09	1.83	5.16	7.35	6.63
1939-40†.....	38.77	0.55	0.61	7.27	1.49
Annual av.‡...	46.21	1.18	3.09	8.29	4.98
Standard deviation of annual average.....		0.71	2.09	2.34	2.61

\*Twelve-month totals averaged for duplicate plots.

†All records for March 1940 were omitted due to drifted snow on plots.

‡The March averages for 1938 and 1939 were used to weight the 1940 figures in making the 3-year average.

The results given in Table 1 are not directly applicable to general field conditions, since variables other than row direction are encountered in the field. However, the data do give an indication of the relative order of magnitude of the effect of contour tillage of corn upon soil erosion losses. Under field conditions where slope lengths greater than those employed in this study are used, the breaking of contours could be expected to occur earlier in the season, which might result in severe soil loss. Further, the close agreement of row direction with the true contour which was obtained under experimental conditions is seldom realized under field conditions.

The general topography of the Northeast exhibits land forms made or modified by glaciation. Under these conditions, the practice of contour tillage on a long slope may introduce the danger of concentration of water due to unavoidable divergence of rows from the contour. Such concentrations may cause soil losses to be more severe in localized portions of a field under contour cultivation than would occur with tillage up and down the slope. For these reasons it is necessary to intercept concentrations of water on long slopes by such conservation practices as strip cropping, terraces, or diversion ditches in order to prevent gullying. However, most of the agricultural land in the Northeast consists of short slopes that are usually convex, so that drainage is seldom concentrated and the largest soil losses occur by sheet erosion with serious gullies being relatively uncommon.

Although interpretation of runoff and erosion data for application to specific field conditions is beyond the scope of this discussion these data may be applied to other conditions by such methods as the treatment for effect of length and degree of slope developed by Zingg (7, 8), and the effect of contour divergence described by Gerdel (1). The effects of contour cultivation on rates of runoff and depressor storage under the conditions of this experiment have been discussed in a previous paper (3).

## CORN YIELDS

Probably the most common measure of acceptability of any cultural practice is crop yield. A comparison of green and oven-dry weights between corn planted up and down the slope in comparison with contour plantings is given in Table 2. The annual yields of each treatment are averages of three plots.

TABLE 2.—*Comparison of average annual yields between corn silage drilled with the slope and on contour.*

Year	Rain June 1 to Aug. 15, inches	Drilled on contour, tons per acre		Drilled with slope, tons per acre	
		Green	Oven-dry	Green	Oven-dry
1937.....	7.56	15.67	3.56	13.81	2.99
1938.....	18.85	12.37	2.50	9.81	1.85
1939.....	5.80	10.34	2.86	9.37	2.55
1940.....	7.85	7.11	1.70	7.19	1.85
Annual av.....		11.37	2.66	10.00	2.31
Standard deviation of annual av. of dry yields.....			0.72		0.54

It was determined from an over-all test by analysis of variance that corn yields on a dry-weight basis under the two methods of planting differ significantly. However, the treatments did not give the same relative results every year. The analysis of variance shows that this difference in effect from year to year, or interaction, is significant. Comparison between treatment effects and interaction indicate that one cannot be certain that contour planting and cultivation of corn will be superior to planting with the slope under all conditions. This might be illustrated by the corn yields for 1940 when soil fertility, after 3 years of continuous corn, had declined to such a low level under both systems of planting that soil fertility was tending to become the limiting factor rather than soil moisture, since soil moisture conditions were about the same as for the year 1937 when the highest corn yield was obtained. Corn yields under both systems at planting must be considered in relation to seasonal effects as well as declining soil fertility.

Relatively high yields of silage corn are generally obtained the first year after plowing under a heavy sod. Nutrients released through the decomposition of the plant residue seemingly contribute largely to this effect. If yield of corn produced may be used as the index, it would seem that contour tillage results in more favorable conditions for this release of plant nutrients than does planting with the slope. The yield records for the last three years indicate the inadvisability of growing corn more than one year in succession on the same piece of land. It might also be concluded that when corn is to be grown, maximum yields will be obtained by using contour tillage combined with a long rotation.

## SOIL MOISTURE STUDIES

Tensiometer records indicated a higher soil-moisture content under contour planting for 1938. The 1938 season was one of relatively high precipitation, thus there was considerable opportunity for the contour ridges, by acting as reservoirs, to increase the amount of moisture entering the soil. There was an average of 1.26 inches less runoff from the contoured plots than from planting with the slope during the 1938 growing season. It is reasonable to expect that this difference in water loss was at least a contributing factor in the recorded difference in crop yields. The character and distribution of rainfall during the 1939 and 1940 growing season was such that, according to tensiometer records, no significant or prolonged effect upon soil moisture was brought about by the contours. During the respective growing seasons, an average of 0.12 inch of rainfall in 1939 and 0.40 inch in 1940 was conserved by the contours.

Apparently the effectiveness of contour cultivation in increasing soil moisture and the resultant effect on crop yields is largely dependent upon the character and distribution of rainfall during the growing season. When showers of high intensity occur with sufficient frequency during the growing season, the contours permit greater storage of moisture for plant growth. When the rainfall distribution is such that no significant amount of moisture is lost as surface runoff, increased yields from contour cultivation are not necessarily to be expected.

## SOIL NITROGEN AND CARBON

Differences in soil loss under the two methods of tillage as given in Table 1 suggest that the conservation of plant nutrients through decreased soil loss might be a contributing factor to the increased corn yield under contour tillage. Under Kansas conditions, Hide and Metzger (2) found highly significant differences in favor of contour cultivation in total nitrogen and carbon between fields cultivated across the slope as compared with fields tilled with the slope. As an indication of the level of soil fertility, total nitrogen and carbon were determined on all plots at the start of the experiment in 1937 and again in 1940. The results of this study are given in Table 3.

TABLE 3.—*Total nitrogen and total carbon with corresponding soil loss for contour tillage of silage corn vs. tillage with the slope, Soil Conservation Experiment Station, Sussex, N. J.*

Method of planting	Total soil loss, 1937-40 tons per acre	Total nitrogen, lbs. per acre			Total carbon, lbs. per acre		
		1937	1940	Difference	1937	1940	Difference
With slope	24.9	3,680	3,200	-480	36,800	32,800	-4,000
On contour	3.6	3,920	3,440	-480	39,200	38,600	-600

Because of sampling and analysis errors, only figures greater than  $\pm 2,300$  pounds of carbon and  $\pm 100$  pounds of nitrogen can be con-



sidered significant. The maximum loss of total carbon occurred on the plots with the highest soil loss. This is as might be expected, but it is difficult to explain the uniformity in total nitrogen decrease under both methods of tillage, for one might expect nitrogen losses to follow a trend similar to the carbon losses. It is suggested that more favorable moisture conditions, as was indicated by the tensiometer readings, and greater moisture movement through the soil under contour tillage as reflected by runoff records may be related to the nitrogen decrease under contour planting. A more uniform moisture condition should stimulate greater microbiological action, which might result in the liberation of nitrogen that could be used for crop growth, or with no crop present on the land might be further transformed or lost by leaching. In this connection the importance of using cover crops to take up and conserve for the next crop this probably available nitrogen, as well as provide soil protection during the fall and winter months, cannot be overemphasized.

In view of the fact that nitrogen and carbon changes are based on analyses of the plot soil and not on analyses of eroded material, their interpretation is limited to indicating that both systems of tillage, when followed for this period of time, are exhaustive of soil fertility. Further information on these differences might be obtained from a study of the eroded material. In contrast with the losses of nitrogen and carbon on the silage corn plots, it is of interest to point out that adjacent plots with identical previous land use history, except that they were seeded to a grass-legume mixture in 1937 when the corn plots were planted to corn, showed a gain of 180 pounds of total nitrogen and 4,000 pounds of total carbon per acre for the 4-year period.

#### SUMMARY AND CONCLUSION

Soil and water losses, silage corn yields, soil-moisture tension conditions, and changes in total nitrogen and carbon are presented for planting on the contour vs. planting with the slope on Dutchess loam soil in northern New Jersey.

Soil losses for a 3-year period of 3.6 tons per acre under contour planting compared with 24.9 tons per acre under up-and-down-hill planting were found to be highly significant. Corn planted on the contour on the same land for 4 years in succession produced an average annual yield on the green weight basis of 11.37 tons per acre, while plots planted up and down hill gave a yield of 10.00 tons. Tensiometer readings suggested a probable relationship between increased moisture content under contour tillage and higher silage corn yields.

Soil nitrogen and organic matter showed a marked decrease during the period of study under both systems of management. Decreases in soil nitrogen indicate the importance of using a cover crop in connection with the efficient utilization of nitrogen under both systems of management as well as for providing soil protection during the vulnerable winter months.

In order to obtain maximum conservation of soil resources and highest yields, silage corn should be planted on the contour and

limited to land in a long grass or legume rotation. The growing of corn more than 1 year in succession on the same land is to be discouraged.

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## NATURAL SELECTION IN VARIETAL MIXTURES OF WINTER WHEAT<sup>1</sup>

H. H. LAUDE AND A. F. SWANSON<sup>2</sup>

THE importance of natural selection among plants has long been recognized, but the rate at which such changes may occur in cultivated crops has only recently received attention. Harlan and Martini<sup>3</sup> determined the rate of natural selection in a mixture of 11 easily recognized varieties of barley which was studied at 10 stations for a period of 4 to 12 years. They found that at all stations there was a rapid elimination of the less-adapted sorts. At most places the variety that would eventually dominate the population was quickly evident. The leading variety varied with the location of the station. A variety dominant at one station in some cases was eliminated at another. Few varieties survived at all stations. Some varieties increased for a time and then decreased.

This paper is a report on the rate at which changes in varietal ratios occurred in successive crops after two varieties of winter wheat were mixed in the proportion of equal numbers of kernels. One mixture included Kanred and Harvest Queen, the other Kanred and Currell.

One portion of each mixture was sown at Manhattan, Kans., and another at Hays, Kans., in the fall of 1931. Seed from the previous crop was used to sow each succeeding crop at the respective station so that the changes in the varietal ratios of the population were cumulative for each station. The experiment was continued for 7 years at Hays and 9 years at Manhattan. Since Harvest Queen and Currell are awnless, there was no difficulty in distinguishing these varieties from Kanred. The rate at which natural selection occurred was determined on the basis of separations of the plants which were pulled from sample areas. The number of heads and number of kernels of each variety were determined in the tests at Manhattan and the number of heads and weight of grain at Hays.

### CHANGES IN VARIETAL PLANT RATIOS

The cumulative changes in the percentage of plants that survived in the varietal population when Kanred was grown in competitive mixtures with Harvest Queen and with Currell are shown for both Manhattan and Hays in Table 1.

<sup>1</sup>Cooperative investigations of the Kansas Agricultural Experiment Station, Fort Hays Branch Experiment Station, Hays, Kans., and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture. Contribution No. 328, Department of Agronomy, Kansas Agricultural Experiment Station, and No. 34, Fort Hays Branch Experiment Station. Received for publication December 8, 1941.

<sup>2</sup>Agronomist, Kansas Agricultural Experiment Station, and Associate Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, respectively.

<sup>3</sup>HARLAN, H. V., and MARTINI, M. L. The effect of natural selection in a mixture of barley varieties. Jour. Agr. Res., 57:189-200. 1938.

TABLE 1.—Percentage of plants of wheat varieties grown in competitive mixtures with Kanred at Manhattan and Hays, 1932 to 1940.

Year	Manhattan		Hays	
	Harvest Queen	Currell	Harvest Queen	Currell
1932.....	46	66	49	47
1933.....	56	58	48	31
1934.....	39	52	25	15
1935.....	31	31	26	8
1936*.....	26	18	—	—
1937.....	19	8	16	1
1938.....	15	6	10	2
1939*.....	—	—	—	—
1940.....	6	2	—	—

\*Percentage of plants not determined in 1936 at Hays and 1939 at Manhattan.

The rate of change was so rapid in both mixtures as to shift the varietal ratios from equal proportions of the two paired varieties to nearly pure stands of Kanred at both stations in less than 9 years. At Hays, the Kanred plants gained in population over both Harvest Queen and Currell from the first year, while at Manhattan the number of Harvest Queen and Currell plants exceeded the number of Kanred plants for 2 and 3 years, respectively, after which Kanred gained rapidly over the other variety in each mixture.

#### CHANGES IN THE VARIETAL KERNEL RATIOS

At Manhattan a study was made of the cumulative changes which took place from year to year in the varietal ratios of number of kernels in the populations as shown in Table 2.

TABLE 2.—Percentage of number of kernels in wheat varietal mixtures grown at Manhattan.

Year	Kanred	Harvest Queen	Kanred	Currell
1931*.....	50	50	50	50
1932†.....	—	—	—	—
1933.....	53	47	54	46
1934.....	63	37	60	40
1935.....	70	30	82	18
1936.....	79	21	88	12
1937.....	83	17	94	6
1938.....	92	8	96	4
1939.....	90	10	99	1
1940.....	93	7	99	1

\*Original mixture.

†Kernel percentages were not determined in 1932.

There was a consistent progressive decline in the proportion of Harvest Queen and Currell in the respective mixtures. Kernel counts were not made of the populations at Hays, the ratios of the grain produced having been determined by weight.

## HOW COMPETITION CHANGED THE VARIETAL RATIOS

Harlan and Martini, in their studies of natural selection in mixtures of barley varieties, observed that "the number of plants of a given variety that will be present in any year will depend on the number (not the weight) of seeds sown, and on the percentage of survival of the seedlings in competition."

Similarly, the increase of Kanred in mixture with either Harvest Queen or Currell seems to have been brought about by the capacity of this variety to crowd out plants of the less well-adapted varieties and by the greater productivity of the surviving Kanred plants. Evidence of this is shown in Table 3 from data secured at Manhattan for the 5-year period, 1934-38.

TABLE 3.—*Competitive capacity of Kanred in varietal mixtures grown at Manhattan.*

Year crop was harvested	Increase in percentage ratio of Kanred over Harvest Queen in			Increase in percentage ratio of Kanred over Currell in		
	Plants	Kernels	Total	Plants	Kernels	Total
1934.....	8	2	10	-6	12	6
1935.....	6	1	7	9	13	22
1936.....	4	5	9	0	6	6
1937.....	2	2	4	4	2	6
1938.....	2	7	9	0	2	2
Increase in percentage ratio: 1934-38.....	22	17	39	7	35	42

In 1934, for example, a 10% change in favor of Kanred took place in the mixture of Harvest Queen and Kanred. The studies showed that four-fifths, or 8 of the 10% change, was due to the relative increase in number of Kanred plants compared to the varietal ratio in the seed planted. One-fifth of the change was due to the greater number of kernels produced by the Kanred plants. The proportion of Kanred was 39% higher in the crop harvested in 1938 than in the seed planted in the fall of 1933. Of this change, 22% was the accumulated difference due to the higher ratio of Kanred among the plants grown to maturity than in the seed planted the preceding fall, and 17% was due to the higher seed production of the Kanred plants compared to the Harvest Queen plants.

In like manner, the increase in the ratio of the Kanred kernels in the Currell mixture during the same period amounted to 42% of which only 7% represented the change in varietal ratios of plants compared to seed planted. Thirty-five per cent or five-sixths of the total change was due to the capacity of Kanred to produce a larger number of kernels per plant than did Currell. Thus Kanred gained in competition with Harvest Queen principally during the vegetative period, whereas the gain over Currell took place mainly during the fruiting period. It is recognized that Kanred is more winterhardy

than Harvest Queen and much more hardy than Currell. The change reported apparently should not be attributed to this factor since freezing injury was not important in any case during these experiments.

At Hays, the competition by Kanred depressed the proportion of plants, heads, and weight of grain of both Harvest Queen and Currell throughout the experiment. The rate of the decline for Harvest Queen and the corresponding rise of the Kanred population is shown graphically in Fig. 1.

For the first two years, the proportion of Harvest Queen plants was only from 2 to 4% below that of Kanred, but from the third year the fall was very rapid. The number of heads and the weight of the grain (kernel counts not taken) followed closely the same pattern as the ratio of the plant population. The graph also indicates that at Hays the Harvest Queen plants could not compete with the Kanred plants in the production of either heads or grain. Similar changes were observed in the mixture of Kanred and Currell.

#### DISCUSSION

These experiments show that changes in ratios of varieties of wheat when grown in competition may occur during the vegetative stage, the fruiting stage, or both stages. The superior variety may make most of its gain during one of these stages and little if any during the other. Furthermore, the data suggest the possibility of a variety being superior in competition with another during one stage but inferior during the other, the result of which would tend to equalize the effect of competition for the entire life cycle.

Farmers frequently claim that seed stocks if grown continuously will "run out". From the data presented it would seem that a relatively pure variety, if fairly well adapted to a region and not attacked by diseases or contaminated by mechanical mixtures, should, over a period of years, improve rather than deteriorate.

In an unselected population of hybrid material natural selection resulting from plant competition would probably play an important role in determining the strains in the final population. In a mixed genetic population competition may sometimes reduce types which when grown among themselves would be among the better in yield. This might be true of high-yielding dwarf strains which in competi-

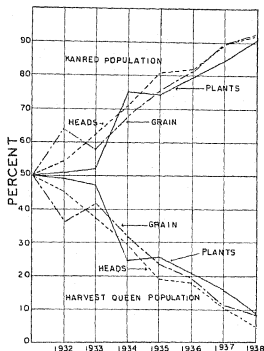


FIG. 1.—Shift in Kanred-Harvest Queen population, Hays, Kans., 1932-38.

tion would suffer from shading by taller types. Such observations have been noted in barley and sorghum.

#### SUMMARY

Studies were made of cumulative changes which took place from year to year in a winter wheat varietal population when Kanred was mixed with Harvest Queen and with Currell.

The rate of change was so rapid in each case as to shift the varietal ratios from equal proportions of the two paired varieties to nearly pure stands of Kanred in less than nine years.

Changes in varietal ratios seem to have been brought about by competition among plants resulting in the survival of a larger proportion of the better adapted variety than of the less well-adapted variety and by the production of more seeds to the plant by surviving plants of the better adapted variety.

# REGISTRATION OF VARIETIES AND STRAINS OF OATS, XI<sup>1</sup>

T. R. STANTON<sup>2</sup>

THE tenth consecutive annual report (7)<sup>3</sup> on the registration of improved varieties of oats was published in March 1941. Since that time three additional varieties, listed and described in the paragraphs below, were approved for registration as follows:

Group and Varietal Name	Reg. No.
Early red:	
Otoe.....	98
Early yellow:	
Tama.....	99
Midseason white:	
Marida.....	100

## OTOE, REG. NO. 98

Otoe (C. I. 2886 and Nebr. 518)<sup>4</sup> originated as one of several hundred selections from commercial Burt oats made by Arthur Anderson and T. A. Kiesselbach of the Nebraska Agricultural Experiment Station at Lincoln in 1920 (1).

Otoe, first known as "Original Strain 13" and later as "Nebraska 518", was tested in nursery rows from 1921 to 1928, and was advanced to field plots in 1929. The application for the registration of Otoe was submitted by K. S. Quisenberry and T. A. Kiesselbach.

The superior characters of Otoe are early maturity, resistance to stem rust, high yield, and stronger straw than Brunner (8). Otoe is classified as an early red oat belonging to *Avena byzantina*. It is of medium height, with yellowish red, rather long, slender grains, many of which carry awns.

The annual and average yields obtained from Otoe and three standard varieties grown in field plots in 1931, 1932, and 1935 to 1941, inclusive, are given in Table 1. For further information on Otoe, see Anderson and Kiesselbach (1), Kiesselbach, et al. (2), and a mimeographed report by Coffman.<sup>5</sup>

## TAMA, REG. NO. 99

Tama (C. I. 3502, Iowa Selection 35-548) was originated (5) from a cross between Victoria and Richland oats made in the green-

<sup>1</sup>Registered under a cooperative agreement between the Bureau of Plant Industry, U. S. Dept. of Agriculture, and the American Society of Agronomy. Received for publication December 22, 1941.

<sup>2</sup>Senior Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, member of the 1941 Committee on Varietal Standardization and Registration, charged with the registration of oat varieties.

<sup>3</sup>Reference by number is to "Literature Cited", p. 279.

<sup>4</sup>C. I. refers to accession number of the Division of Cereal Crops and Diseases, U. S. Dept. of Agriculture.

<sup>5</sup>COFFMAN, F. A. Results from the cooperative coordinated oat breeding nurseries for 1940 and the uniform winterhardness nurseries for 1940-41. U. S. Dept. Agr., Bur. Plant Ind., Div. Cereal Crops and Diseases (Unnumb. Pub.). Oct. 24, 1941. (Mimeographed.)



TABLE I.—*Yields of Otee, Burt, Kherson, and Loggold oats at Lincoln, Neb., 1931, 1932, and from 1935 to 1941, inclusive.*

Variety	C. I. No.	Acre yield, bushels									
		1931	1932	1935	1936	1937	1938	1939	1940	1941	9-yr. av.
Otoe.....	2886	64.0	69.2	43.4	25.2	37.4	68.6	26.5	24.0	60.8	46.6
Burt (Common).....	293	63.9	54.3	36.5	22.7	37.0	62.8	28.1	20.5	57.0	42.5
Kherson (Common).....	459	56.4	61.3	32.3	17.8	36.1	54.6	26.1	19.6	47.0	39.0
Loggold.....	2329	59.9	69.1	40.1	17.5	33.2	56.4	22.7	17.2	50.0	40.7

house at the Arlington Experiment Farm, Arlington, Va., by T. R. Stanton in the spring of 1930. The  $F_1$  generation was grown at Aberdeen, Idaho. The  $F_2$  generation was grown at the Iowa Agricultural Experiment Station, Ames, Iowa, where it was subjected to crown and stem rust developed by inoculating susceptible "rust spreaders" growing adjacent to the  $F_2$  plants. A total of 500 plants was harvested, and an  $F_3$  generation from each of these was grown at Ames in 1932, the plants being again subjected to the two rusts. This generation also was inoculated with loose smut and susceptible segregates eliminated. At harvest, the seed from certain plants from some of these  $F_3$  lines was retained at Ames, and the seed from certain other plants from sister lines was sent to Arlington, Va., for sowing in the greenhouse in the fall of 1932. The Boone and Vicland varieties were developed from the latter stocks (5, 6, 7, 9, 10).

From the seed retained at Ames, successive plant populations were grown and tested for resistance to the rusts and smuts in both greenhouse and field at Ames. Tama was last selected in the  $F_4$  generation in 1935, when it was grown in panicle row 548, and was tested for yield in a single row in 1936. It was tested in replicated nursery plots at Ames from 1937 to 1941; at Kanawha, Iowa, from 1938 to 1941; and at Conesville, Iowa, in 1940. Tama was developed cooperatively by the Iowa Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture. Those having a part in its breeding are H. C. Murphy, L. C. Burnett, T. R. Stanton, and F. A. Coffman.

The application for the registration of Tama was made by H. C. Murphy at the request of the Seed Distribution Committee of the Iowa Agricultural Experiment Station. Tama is an early common oat with a short, stiff straw, and plump, yellow kernels with few awns.

The superior characters of Tama are early maturity, high yield, and satisfactory resistance to nearly all the physiologic races of crown rust, stem rust, and the smuts of oats. It is similar to Boone in all characters but may be slightly superior in yield.

The annual and average yields of Tama in replicated nursery plots at Ames and Kanawha and in the Iowa standard community trials, as compared with those of Boone, Marion, Richland, and Iogold oats in Iowa, are given in Table 2. For further information on Tama, the reader is referred to mimeographed reports by Burnett<sup>6</sup> and Coffman.<sup>7</sup> Tama is being distributed for sowing on Iowa farms in the spring of 1942.

#### MARIDA, REG. NO. 100

Marida (C. I. 2571) was originated from a cross between Markton and Idamine oats made by G. A. Wiebe at the Aberdeen Substation, Aberdeen, Idaho, in 1923. Numerous selections from this cross were tested for resistance to smut at Aberdeen from 1924 to 1928. Seed

<sup>6</sup>BURNETT, L. C. Information from experiments in progress. Small grains summary. Iowa Agr. Exp. Sta. Leaflet F. C. 14, 1941. (Mimeographed.)

and DYAS, E. S. Standard community grain trials. Iowa Agr. Exp. Sta. Leaflet F. C. 13, 1941. (Mimeographed.)

<sup>7</sup>*Loc. cit.*

TABLE 2.—Yields of Tama and four other varieties of oats at Ames and Kanawha, Iowa, and in 91 Iowa standard community trials.

Variety	C. I. No.	Acre yield, bushels										Av. 1939-41 stand- ard com- munity trials
		1937		1938		1939		1940		1941		
		Ames	Kana- wha	Ames	Kana- wha	Ames	Kana- wha	Ames	Kana- wha	Ames	Kana- wha*	
Tama.....	3502	92.8	104.7	65.6	85.3	55.6	85.7	85.3	78.9	46.0	77.8	56.1
Boone.....	3305	96.8	102.3	70.6	70.0	44.7	79.9	83.1	78.3	51.6	75.3	55.4
Marion.....	3247	101.6	97.3	46.6	70.9	54.1	79.9	84.8	76.1	53.8	73.9	54.2
Richland (Iowa 105).....	787	73.1	79.3	41.9	37.2	44.7	65.4	70.6	69.5	18.5	55.6	47.3
Idagold.....	2329	71.2	74.6	37.8	27.8	40.2	69.5	76.9	72.6	23.2	55.5	—

\*Nursery at Ames destroyed by hail in 1941.

TABLE 3.—Yields of Marida and the two parental varieties of oats at Moscow, Idaho, 1920-1940.\*

Variety	C. I. No.	Acre yield, bushels													Average
		1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940		
Marida.....	2571	54.4	86.2	73.3	114.2	103.1	78.0	87.5	86.6	113.0	54.3	84.6	81.5	84.7	
Markton.....	2653	80.6	83.4	66.6	116.8	85.3	66.3	74.1	66.3	133.0	41.5	66.6	79.2	80.0	
Idamne.....	1834	71.4	69.6	59.4	114.6	98.5	57.4	78.1	69.7	106.6	47.0	72.2	99.8	78.7	

\*For further information on Marida see Klages (3) and Michels (4) and footnote 5.

of some of the more promising selections was sent to the Idaho Agricultural Experiment Station at Moscow in 1929. Selection C. I. 2571, which proved to be outstanding for yield and quality there, was named Marida and distributed in 1940 to farmers on the non-irrigated lands of northern Idaho.

Marida was developed cooperatively by the Idaho Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture. Those having a part in the breeding of Marida are C. A. Michels (deceased), K. H. Klages, T. R. Stanton, F. A. Coffman, G. A. Wiebe, L. L. Davis, A. E. McClymonds, and V. F. Tapke. Application for the registration of Marida was made by K. H. Klages.

Marida is an early to midseason white oat of the Silvermine type, with resistance to most of the physiologic races of the oat smuts which occur in the northwestern states.

Michels (4) reported that, "Marida is superior to its parents not only from the standpoint of greater yielding capacity but also from the standpoint of having a stiffer and shorter straw\*\*\*\*. Marida is an early-maturing oat with a stiff straw, a white kernel, and has a high per cent of berry to hull\*\*\*\*."

Marida has been tested in replicated nursery plots at Moscow since 1929. The annual and average yields obtained are given in Table 3.

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REGISTRATION OF IMPROVED SORGHUM VARIETIES, III<sup>1</sup>R. E. KAPER<sup>2</sup>

ONE improved variety of sorghum was approved for registration in 1941 under the name of Norkan, Reg. No. 79.

*Description.*—Plants mid-early, mid-tall; stems slender, juicy and sweet; tillers freely. Ten to 12 mid-wide leaves with cloudy midrib. Sheaths overlapping. Panicles erect, mid-compact cylindroid with slightly tapering apex. Glumes straw to reddish color, ovate shape with sharp tip, covering one-fourth to one-third seed surface. Lemmas awnless. Seed white with reddish spots, small to mid-size, slightly flattened ovoid shape; nucellar layer absent. Threshes freely. Endosperm starchy and corneous.

Norkan is an early maturing forage sorghum originated and distributed from the Fort Hays Experiment Station, Hays, Kans. It is a selection from a hybrid between Atlas sorghum and Early Sumac. The cross was made by Dr. J. H. Parker in 1926 and the final selection was made by A. F. Swanson in 1938. Seed of the new variety was increased and distributed for the first time in 1941 when it was approved for certification by the Kansas Crop Improvement Association.

Norkan has the earliness of Early Sumac and the white grain of the Atlas parent. This white-seeded early maturing variety of sweet sorghum is adapted for areas north of the latitude where Atlas is grown.

Comparative yields of Norkan with Atlas and Early Sumac, the two parents, tested at Hays, Kans., during the 5-year period from 1936 to 1940 are given in Table 1.

TABLE 1.—*Yields of forage in tons per acre of Norkan and other varieties of sorgo tested at Hays, Kans., 1936-1940, inclusive.*

Variety	Year					Av.
	1936	1937	1938	1939	1940	
Norkan.....	1.25	1.47	2.28	1.99	1.77	1.75
Atlas.....	1.49	1.59	3.10	1.42	2.51	2.02
Early Sumac.....	1.16	1.68	2.53	1.51	1.71	1.72

<sup>1</sup>Registered under cooperative agreement between the Bureau of Plant Industry, U. S. Dept. Agriculture, and the American Society of Agronomy.

<sup>2</sup>Agronomist in Charge Sorghum Investigations, Texas Agricultural Experiment Station, Lubbock, Texas. Member of the 1940 Committee on Varietal Standardization and Registration of the Society charged with the registration of sorghum varieties.

# BARLEY VARIETIES REGISTERED, VII<sup>1</sup>

H. K. HAYES<sup>2</sup>

ELEVEN varieties of barley have been registered previous to this report. Three varieties were approved for registration in 1940.<sup>3</sup>

## BEECHER, REG. NO. 12

Beecher was produced from a cross of Atlas×Vaughn made in 1927 and was first distributed commercially in 1940. It is a new dry-land barley of considerable promise for the highland section of the Central Great Plains. It is a six-rowed, smooth-awned variety, with rough awns at the tips, and of erect habit of spike as in the Club Mariout variety. The straw is slightly coarser than Club Mariout, the height of plant is about the same as Club Mariout, and the hull is thinner. In field plot trials since 1938, Beecher has averaged 40.5 pounds weight per measured bushel as compared with 37.3 pounds for Club Mariout. Yield trials are reported in Table 1.

The names of breeders and introducers are V. H. Florell, J. J. Curtis, and J. F. Brandon, the new variety being developed and introduced cooperatively by the U. S. Dept. of Agriculture and the Colorado Agricultural Experiment Station.

TABLE 1.—Comparative yields in bushels per acre of Beecher and Club Mariout in nursery trials with three replications and plot trials with four replications, at Akron, Colorado.

Variety	Yield in bushels per acre					
	Nursery		Field plots			Average
	1936	1937	1938	1939	1940	
Beecher, C. I. 6566.....	58.8	37.0	42.5	13.9	2.4	30.9
Club Mariout, C. I. 261.....	42.9	32.3	30.9	10.7	1.9	23.7

## LICO, REG. NO. 13

Lico was produced from a cross of Coast×Lion made in 1922 and the variety was first distributed in 1937. It is a stiff-strawed, smooth-awned, six-rowed variety with a medium lax head, which stands erect, resembling the Coast parent. The rachilla hairs are short and the aleurone is colorless. Yield trials are reported in Table 2.

<sup>1</sup>Registered under a cooperative agreement between the Bureau of Plant Industry, U. S. Dept. of Agriculture, and the American Society of Agronomy. Received for publication February 23, 1942.

<sup>2</sup>Chief, Division of Agronomy and Plant Genetics, Dept. of Agr., Univ. of Minn., St. Paul, Minn. Member of committee on Varietal Standardization and Registration of the Society charged with the registration of barley varieties.

<sup>3</sup>HAYES, H. K. Barley varieties registered, VI. Jour. Amer. Soc. Agron., 33:252-254. 1941.

The variety was bred by D. W. Robertson and introduced by the Colorado Agricultural Experiment Station.

TABLE 2.—*Comparative yields in bushels per acre of Lico and Trebi in nursery trials with 10 replications and field plot trials with three replications at Fort Collins, Colorado.*

Variety	Yield in bushels per acre								Average
	1932	1933	1934	1935	1936	1937	1938	1940	
Lico, C. I. 6279.....	89.4	57.4	74.9	93.9	81.4	75.8	63.1	51.4	73.4
Trebi, C. I. 936.....	81.2	66.3	78.1	88.5	58.8	58.4	58.4	59.9	68.7

#### TEXAN, REG. NO. 14

The new variety is intermediate in growth habit between true winter and true spring barley varieties and will produce from either spring or fall seeding under Texas conditions. It is less hardy than Tennessee Winter, but about equal to Wintex and slightly earlier in maturity than Wintex. It is recommended for fall seeding in the large, blackland area of central Texas. Yield trials are reported in Table 3.

TABLE 3.—*Comparative yields of Texan and other varieties at the Temple Substation, Temple, Texas.*

Variety	Yield in bushels per acre				
	1938	1939	1940	1941	Average
Texan, C. I. 6499.....	22.8	41.0	20.4	22.8	26.8
Wintex, C. I. 6127.....	13.0	37.3	19.4	22.0	22.9
Finley, C. I. 5901.....	7.2	32.0	19.2	22.1	20.1
Tennessee Winter, C. I. 6125.....	22.2	20.0	20.6	18.9	20.4
Tennessee Winter 61, C. I. 3545.....	24.0	28.6	23.2	18.5	23.6
Missouri Early Beardless C. I. 6051.....	15.9	18.3	15.0	14.6	16.0
Tennessee Beardless 5, C. I. 3384.....	11.4	19.2	15.0	14.5	15.0

It is a six-rowed, smooth-awned variety and is resistant to mildew and moderately resistant to net blotch under field conditions.

Texan barley was produced from a selection made in 1933 by I. M. Atkins from Composite Cross C. I. 5530 of winter barley made by H. V. Harlan. It was developed cooperatively by the Texas Agricultural Experiment Station and the U. S. Dept. of Agriculture.

## NOTES

### DANDELION CONTROL WITH DICHLOROETHYL ETHER<sup>1</sup>

IN the last few years a number of papers have been published on 2, 2' dichloroethyl ether as a soil insecticide. Though in some of these mention was made of injury to certain crop plants, no one reported injury to any common weeds. While investigating the control of white grubs, *Phyllophaga* spp., in Kentucky bluegrass pastures during the summer of 1941, the junior writer observed definite injury to dandelions, *Taraxacum palustre* (Lyons) Lam. and DC. var. *vulgare* (Lam.) Fern., on plots treated with 2, 2' dichloroethyl ether solution.

On August 29, 1941, a series of 24 plots, each 6 feet square, was established on Kentucky bluegrass sod having an average of 13 dandelions per square yard. The grass had not been mowed or grazed for several years. Each of five treatments was applied to four randomized plots and four randomized plots were left untreated. Eleven days after treatment there was a mean "kill" of dandelions of 95.1% on plots receiving  $2\frac{1}{4}$  gallons per square yard of dichloroethyl ether solution containing 72 cc of ether. The same amount of ether solution with the addition of 0.05% or 0.1% of "Tergitol" penetrant 7 did not give significantly different results. On plots receiving 1 gallon of ether solution per square yard containing 32 cc of ether and 0.05% "Tergitol" there was a mean "kill" of 78.6%. This was a significantly poorer "kill" than that obtained with  $2\frac{1}{4}$  gallons of ether solution per square yard.

Dichloroethyl ether at the heavier rate used in this test killed the roots of dandelions to a depth of 4 to 6 inches, the roots at first turning brown, later becoming soft, and finally decaying completely. Nine weeks after the treatments were made young leaves were emerging from the soil from a few very large roots that had been killed 6 inches deep. Dandelion roots of large, vigorous plants, such as were present in this sod, were found to have penetrated as deep as 30 inches. In most plots treated with ether no injury to bluegrass was evident 8 weeks after treatment, but in a few plots small areas of bluegrass turned brown and died. The odor of dichloroethyl ether could be detected for about 3 weeks after treatment.

To test the effectiveness of dichloroethyl ether for controlling buckhorn (*Plantago lanceolata*, L.) eight 6-foot-square plots were laid out in a closely mowed Kentucky bluegrass lawn containing much buckhorn and about 30 dandelion plants per square yard. Four plots were treated on August 30 with  $2\frac{1}{4}$  gallons per square yard of dichloroethyl ether solution containing 72 cc of ether and 0.1% "Tergitol", while four plots were left untreated. Only a very few buckhorn plants were injured 11 days after treatment, while 92.5% of the dandelions appeared to be killed in the treated plots. Three and a half months after treatment only two dandelion plants had developed new leaves above ground.

<sup>1</sup>The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.



Spiny sida (*Sida spinosa* L.) was killed by dichloroethyl ether at the heavier rate. Plants not affected included spotted spurge (*Euphorbia maculata* L.), yellow foxtail (*Setaria lutescens* (Weigel) F. T. Hubb.), and three-seeded mercury (*Acalypha virginica* L.). There was slight injury to plants of *Paspalum pubescens* Muhl.—W. C. TEMPLETON, JR., and P. O. RITCHER, *Kentucky Agricultural Experiment Station, Lexington, Kentucky.*

#### A MODIFIED RESISTANCE BLOCK FOR SOIL MOISTURE MEASUREMENT

THE resistance block developed by Bouyoucos and Mick<sup>1</sup> for measuring soil moisture seems to meet with acceptance as a practical field instrument, but Cummings and Chandler<sup>2</sup> suggest that some variability in readings may be traced to the fact that the electrical current conductance path is partially outside the block. The resistance of the system changes with the conductance of the medium in which the block is placed.

The conductance path may be confined wholly within the block by electrodes of the design shown in Fig. 1. One electrode is located centrally in a cylindrical screen. The screen itself forms the second or outer electrode.

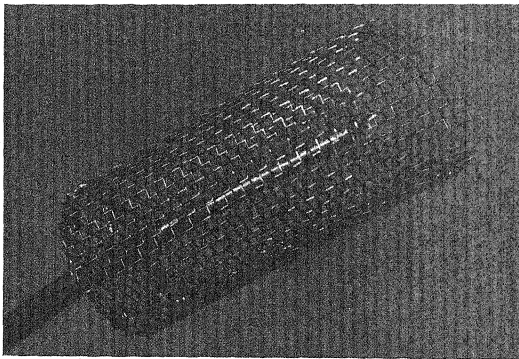


FIG. 1.—View of screen cage electrode with enclosed central electrode.

<sup>1</sup>BOUYOUCOS, G. J., and MICK, A. H. An electrical resistance method for the continuous measurement of soil moisture under field conditions. Mich. Agr. Exp. Sta. Tech. Bul. 172.

<sup>2</sup>CUMMINGS, R. W., and CHANDLER, R. F., JR. A field comparison of the electrothermal and gypsum block electrical resistance methods with the tensiometer method for estimating soil moisture *in situ*. Soil Sci. Soc. Amer. Proc., 5:80-85, 1940.

Experimental blocks or cylinders have been made by casting hydrocal<sup>3</sup> about similar electrode pairs. The electrodes in these cylinders are of copper and the leads are rubber-insulated electric cord. The inner electrode has dimensions of  $\frac{1}{8}$  by  $1\frac{1}{2}$  inches, the outer cylindrical electrode is 1 inch in diameter and  $2\frac{1}{2}$  inches long. Ordinary 14-mesh window screen was used to make the cylindrical electrodes. The completed cylinder is approximately  $1\frac{1}{4}$  inches in diameter and  $3\frac{1}{2}$  inches long. The resistance of the cylinder at saturation is about 350 ohms, irrespective of whether the surrounding medium is water or air.

Some of these cylinders have been under field test for one season with favorable results. The results are no better or no worse than the results obtained from comparable installations of Bouyoucos and Mick blocks, consequently no claim can be made on the basis of present results of any marked improvement in resistance block design. The basis for the changed electrode design is, however, sound. Confinement of the conductance path to the space between the electrodes simplifies the procedures that are involved in critical testing of the blocks. Where a wide variety of conditions must be encountered in the field use of an instrument, the fewer the uncontrolled factors, the more reliable in general are the results obtained.

Since the inner electrode of the system is shielded by the outer electrode, only the outer can serve as a ground on alternating current bridges where one terminal is maintained at ground potential.—C. S. SLATER, *Soil Conservation Service, Maryland Agricultural Experiment Station, College Park, Maryland.*

#### A DIRECT WEIGHING METHOD FOR SEQUENT MEASUREMENTS OF SOIL MOISTURE UNDER FIELD CONDITIONS

A NUMBER of types of instruments have been devised during the last several years for repeated measurement of soil moisture in the field. All the instruments to date, with the exception of the tensiometer which is limited to readings of film tension during relatively wet conditions, approach the problem through use of a porous medium permanently imbedded in the soil. Use of an absorbent foreign material has been found to be a necessary essential in moisture-measurement methodology to enable the establishment of a stable and standardized zone within which to secure the measurements of moisture change.

Departure between methods has been concerned largely with principles and procedures in securing a measure of the amount of moisture in the medium. Electrical resistance, thermal conductance, and dielectric readings have been used as indices of the amount of this moisture. In many cases these values have been found to fluctuate with respect to certain other conditions in the soil environment, as well as moisture change, or to be a function of refinement in equipment design and operation, hence are not always related directly to the actual moisture in the soil.

<sup>3</sup>A commercial gypsum product.

The gravimetric principle, as a direct means of determining the amount of moisture in a porous medium, has received little attention of late. The growing need for a simple means of obtaining field measurements of soil moisture has led to the application of this approach in the instrument here described, with which a direct moisture measurement unaffected by other conditions of environment is obtained.

The instrument consists of a porous chamber, or casing installed in the soil at the depth moisture readings are desired. The casing contains a closely fitting tapered porous cone, or plug, which may be removed through a tube connecting the upper rim of the casing with the soil surface by means of a suitable hook. A somewhat idealized design of the plug and casing with a short section of lift tube attached is shown in Fig. 1. The scale is greater than actual size.

The instrument under present field test conform to the specifications shown in all detail except the use of glass lift tubes, and for convenience in molding, the outer walls of the casings were made

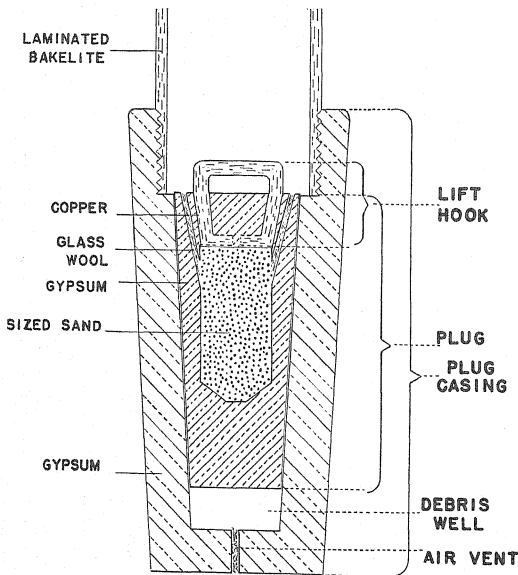


FIG. 1.—Construction of porous well casing and removable plug unit.

vertical instead of tapered. Hydrocal<sup>1</sup> was used in casting the plugs and casings.

The inner portion of the plug contains washed sand properly sized to provide an upper pore-size range corresponding to that in the soil. The plug thus serves as a removable moisture medium responsive to changes in moisture in the given soil from field capacity to permanent wilting point. A nonporous rod suspended from a cap fitted to the top of the lift tube displaces the air from the well and prevents vapor circulation between the plug and the surface.

Moisture content is determined by removing the plug through the lift tube and weighing to 0.01 gram. After weighing, the plug is returned to the well and tapped snugly in place to eliminate interface. Suitable spring scales suspended by hand or incased torsion balances may be used in weighing. Evaporation loss during weighing is relatively unimportant. The range in weight of the plug from dryness to saturation is approximately 5 grams. Since the plugs are of standard construction, pF equivalents to the weight readings may be obtained by a laboratory calibration. A plug also may be calibrated in the field for each soil type in terms of moisture per cent or volume.

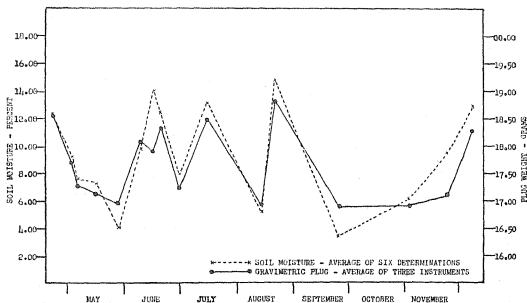


FIG. 2.—Comparison of gravimetric plug readings and soil moisture determinations.

Daily field readings of soil moisture for a period of 7 months made with instruments as here described have shown promising results through the range thus far experienced. The degree to which moisture change is reflected by the gravimetric plug readings is seen in Fig. 2. The data were taken during the 1941 field season.—WENDELL E. DAVIS and C. S. SLATER, *Soil Conservation Service, Maryland Agricultural Experiment Station, College Park, Md.*

<sup>1</sup>A commercial gypsum product.

NOTES ON SUBSURFACE LATERAL MOVEMENT OF WATER  
APPLIED TO EXPERIMENTAL AREAS

RECENTLY in this JOURNAL<sup>1</sup> a method of applying water to an experimental area was described in which it was implied that subsurface lateral movement of water was satisfactorily controlled. Using an 8-inch-diameter ring, located within a 16-inch-diameter ring, which in turn was located within a 24-inch wetted square, it is stated that "lateral movement of water from the central ring was either completely eliminated or greatly diminished." It is said that tubes 14 inches long inserted in the soil "gave good measurement of the initial intake of water into the soil and of the relative permeability of the soil core enclosed within the cylinder, but could not be relied upon to give quantitative information regarding the permeability and the percolation rate of the entire soil profile."

This conclusion is not surprising, but the problem apparently is broader than that indicated. It may be noted in passing also that the particular method referred to is one that we have not used in our work of the past three years, except in a comparative way. It will be seen from the detailed data in a forthcoming report, however, that certain limitations are to be found in each of the different methods thus far developed for measurement of infiltration.

Evidently the authors were interested in percolation rates rather than infiltration rates, and their procedures have deviated considerably from those that we have used with this type of equipment. They have reported using 8 to 10 replicates and found high variability. Normally we have used 20 to 24 replicates, because it is recognized that soil is highly variable with reference especially to the internal movement of water. The authors have used tubes 8 by 14 inches in size and applied water apparently for 8 hours or more. We have used,<sup>2</sup> normally, tubes 9 by 18 inches or 9 by 24 inches, depending upon soil characteristics, with the objective of reaching into a less permeable horizon, and have applied water for periods of 3 to 3½ hours. Obviously greater movement of subsurface water would be expected under the former than the latter procedures.

Subsurface lateral movement of water, we believe, occurs to some extent, in certain soils at least, with *any* method of applying water to a small plot. Evidence of this has been found in our work with rainfall simulators of various kinds and sizes, with concentric rings, essentially similar to those reported by the authors, and with tubes. As between the latter two methods, the standard deviation normally has been lower in any series of determinations where the core is encased than in a corresponding series where it is open, as with concentric rings. In a series of experiments recently completed replicated determinations by each of four different kinds of infiltrometers were

<sup>1</sup>NELSON, LEWIS B., and MUCKENHIRN, R. J. Field percolation rates of four Wisconsin soils having different drainage characteristics. Jour. Amer. Soc. Agron., 33: 1941.

<sup>2</sup>FREE, G. R., BROWNING, G. M., and MUSGRAVE, G. W. Relative infiltration and related physical characteristics of certain soils. U. S. D. A. Tech. Bul. 729. 1940. (Page 4.)

made on each of 36 different sites. The comparisons included three different kinds of land use on each of three different soils. The different types of infiltrometers all sorted the land use conditions in the same order, and provided good relative data. It is doubtful, however, whether any of them provided absolute and precise values, because the escape of some subsurface water, through border and buffer areas, probably occurred at least in small amounts in certain soils.

In Fig. 2 of the paper by Nelson and Muckenhirn, the intake of water by Miami silt loam is shown. The curve for this soil indicates that more than 30 inches of water entered the profile of this soil in their experiments. The water-holding capacity as reported ranges from 33.9 to 38.2 for the "plow layer", the B, and the B<sub>2</sub>. Must we assume that all of this water passed downward vertically beneath the experimental area without any lateral movement? In our work with this soil we have found a marked tendency for lateral movement to occur just above the first more dense sub-horizon.

Some measurements of the rate of lateral movement of water in Lordstown stony silt loam were reported in U. S. Dept. of Agriculture Technical Bulletin No. 729. In the work done at that time the technic used did not provide for a wetted border surrounding the experimental area.

TABLE 1.—*Relation of wetted borders to infiltration measurements.*

Site No.	No wetted border, inches per hour	4 x 6 ft. wetted area, inches per hour	8 x 12 ft. wetted area, inches per hour
Falcon Gravelly Sandy Loam (Shallow Phase)			
1.....	0.963	0.619	0.895
2.....	1.026	0.614	1.023
3.....	0.998	0.199	0.105
4.....	0.732	0.571	0.523
5.....	0.437	0.550	0.444
6.....	0.283	0.316	0.408
7.....	0.600	0.436	0.318
8.....	0.989	0.507	0.456
9.....	0.717	0.502	0.520
Means.....	0.749	0.479	0.521
Falcon Gravelly Sandy Loam			
1.....	1.167	0.497	0.414
2.....	0.617	1.095	1.176
3.....	0.755	0.827	0.418
4.....	0.681	0.546	0.948
5.....	0.438	0.405	0.354
6.....	0.775	0.689	0.503
7.....	0.625	0.646	0.335
Means.....	0.723	0.672	0.593
Av. both soils.....	0.736	0.576	0.557

Since that time in our work all plots have been provided with buffer-wetted borders. Even then some lateral movement possibly occurs. Table 1 gives some data obtained from experimental areas 1 by 2½ feet in size, lying within a wetted area that normally was 4 by 6 feet. When the border was protected from wetting, the mean infiltration during the last portion of runs at or near saturation was 0.736 inch per hour. With the normal 4 by 6 feet wetted area, it was 0.576 inch per hour, while it dropped to 0.557 inch per hour when located within a wetted area of 8 by 12 feet. Not all sites showed these effects and it is not implied that they invariably occur, but it is clear that under certain circumstances, which are not unusual in any observable respect, a 4 by 6 feet wetted area was insufficient completely to protect an inner 1 by 2½ feet experimental area.

In brief, two definite questions occur, *viz.*, (1) Was lateral movement "completely eliminated" and indeed can it be even "greatly reduced" in all cases by the described and proposed method? and (2) Can percolation rates be determined in this way?

Certainly it would seem that the rate of intake of water by a soil profile does not necessarily equal the rate of outgo at a lower plane, not only where there is opportunity for some lateral subsurface movement to regions beyond the experimental area, but also where durations of application and depth of profile are varied.

Though considerable work has been reported on the capillary movement of soil moisture, the problem of movement of gravitational water is in great need of further investigation. It is complex and difficult to study because of the dynamic characteristics of soil, yet it is of great importance because it so often exerts chemical and physical effects of considerable magnitude. The authors have pointed out that internal drainage is often a major factor controlling crop adaptation and are to be commended for undertaking so important a study, one which it is hoped they may continue.—G. W. MUSGRAVE, *Soil Conservation Service, U. S. Dept. of Agriculture.*

#### A NOMOGRAM FOR FINDING THE AREAS OF BEAN LEAVES<sup>1</sup>

MANY agricultural research workers have interested themselves in the past few years with finding the areas of leaves of various plants. The areas were found in many instances by use of a planimeter from blue prints or tracings of the leaves. This usually requires a great deal of labor and time.

There are several experiments now being carried out at Michigan State College in which areas of leaves form important parts of the investigations. These pertain to counts of European red mites per leaf area of Delicious apple tree leaves and effectiveness of sprays, effects of sprays in relation to transpiration of sugar beet leaves and honeysuckle leaves, leaf area vs. yield of field beans, etc.

The purpose of this article is to show how the area of a bean leaf, which is composed of three leaflets, can be found quickly by a chart

<sup>1</sup>Journal Article No. 562 (n.s.) of the Michigan Agricultural Experiment Station.

which is called a nomogram (Fig. 1). Davis<sup>2</sup> found in his work with beans that the area of a bean leaf can be found from the following equation:  $A = 0.004517 W \cdot L$ , where  $A$ ,  $W$ , and  $L$  represent, respectively, the area in square inches, the width, and the length of the center leaflet in  $1/20$  inches. To use this equation, it is necessary to find the width and length of the center leaflet, multiply them together, and then multiply this product by the constant  $0.004517$ . This means that it is necessary to record the measurements of the width and length and then later substitute their product into the above equation in the computing room or laboratory. This takes a great deal of time if many leaves are measured. The nomograph will enable one to write the area as soon as the width and length are measured without recording them.

The center leaflet of the bean leaf was measured by laying it on rectangular coordinate paper with 20 vertical and horizontal lines to the inch. In this way the measurements of the length and width can be secured at the same time and to the nearest  $1/20$  inch, which is as accurate as one usually desires. One person can place the leaflet on the grid paper quickly without removing it from the plant and read off the width and length to a second person who can secure at once the area of the entire leaf by placing a straight edge across the nomogram. This area is the only item which is recorded. The amount of labor is reduced to a minimum by using this device for all areas are found in the field without the computation in the laboratory.

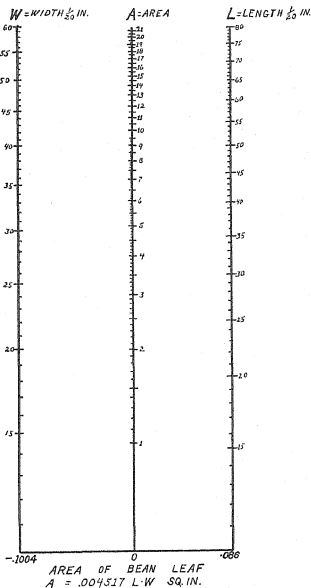


FIG. 1.—Nomogram for finding the area of a bean leaf from the width and length.

<sup>2</sup>DAVIS, J. F. The relationship between leaf area and yield of the field bean with a statistical study of methods for determining leaf area. Jour. Amer. Soc. Agron., 32:323-329. 1940.



The nomogram for finding the area of a bean leaf is shown in Fig. 1, and was obtained from the above equation by using the linear relation between the logarithms of the area,  $0.004517L$  and  $W$  as  $\text{Log } A = \text{Log } 0.004517L + \text{Log } W$ . The scales on each of the axes shown in Fig. 1 were obtained as logarithmic scales for the chosen interval of 9 inches for the heights of the axes of the measurements pertaining to the width and length measurements. Methods for constructing nomograms are given in any text on nomography.

The area of the leaf with length and width of the center leaflet equal, respectively, to 51 ( $1/20$  inch) and 28 ( $1/20$  inch) is found by laying a straight edge on the width axis in Fig. 1 on 28 and on the length axis on 51, and then reading where the straight edge crosses the area axis, which is the center axis. This area value is 6.4 square inches, reading to the nearest 0.1 square inch. The area of a leaf with the length and width of the center leaflet equal, respectively, to 60 ( $1/20$  inch) and 40 ( $1/20$  inch) is also found by laying the ruler or straight edge on the length axis on 60 and the width axis on 40; the area read from the area axis is 10.7 square inches.

The nomogram is of little value unless one is going to measure many leaves, or is going to carry on the study with the same kind of leaves at least two years, or when the equation for finding leaf area in terms of length and width measurements does not hold from season to season. However, if the investigation is going to extend over several years and if the equation obtained from measurements the first years is suitable for the measurements of the same kind of leaves the succeeding years, then the nomogram constructed from the first year measurements will be beneficial in all future work. Davis has been using the above equation during several seasons, and in his case the nomogram will greatly reduce the amount of time and labor devoted to finding leaf areas. Frequently, information is gathered the first year or for the first 400 or 500 leaves for determining the areas of all future leaves. In such cases it would pay to construct a nomogram from the first set of data and use it for future work.

This article was written for the purpose of calling attention to this very handy method of finding the area of leaves. Similar methods might be used for other relations. The nomogram for obtaining areas of bean leaves will not be applicable for other kinds of leaves.—  
WILLIAM DOWELL BATEN, *Department of Mathematics, Michigan State College, and Research Associate in Statistics, Michigan Agricultural Experiment Station, East Lansing, Mich.*

#### OAT VARIETIES FOR WINTER PASTURE PRODUCTION<sup>1</sup>

THE rapid increase in the use of oats for winter pasture brings to light the need for research to show the relative value of different varieties of oats for this purpose. No data of this character seem to be available.

<sup>1</sup>Contribution from the Department of Agronomy, Agricultural and Mechanical College of Texas and the Texas Agricultural Experiment Station, College Station, Texas. Contribution No. 1418 of the Texas Agricultural Experiment Station, Cooperative Project No. 481.

The oat varieties we now have are the result of many years of breeding and selection for grain production. The varieties or strains which did not prove to be good grain producers have been gradually eliminated.

The qualities thought to be most desirable in an oat variety for winter pasture production are not the same as those given most attention when selecting a variety for grain production. Therefore, retesting the present oat varieties to determine their value for winter pasture production seems worthwhile. This is being undertaken by the Department of Agronomy of the Agricultural and Mechanical College of Texas.

The quantity, quality, and timeliness of vegetative material produced, as opposed to total bushels of grain matured, are among the dominant considerations when evaluating an oat variety for winter pasture production. To determine these an entirely new approach to the problem is required.

In two years of work on this problem at the Agricultural and Mechanical College of Texas several points have been brought out which may be of interest to agronomists and geneticists interested in small grains and winter pasture. Among the more significant of these are the following:

1. In 1939-40 the low-yielding variety Lee×Victoria, Ark., 2-25, yielded 7,231 pounds of green matter. The high producer, Lee C. I. 2042, gave 13,705 pounds of green matter, or nearly twice as much.
2. For pasture production the determination of the differences in percentage of air-dry material is significant. In 1939-40 the lowest percentage in air-dry matter was from Nortex×Victoria M 19-17 with 16.8%. The highest was Nortex×Berger M 5-3 with 22.1% air-dry matter. This difference of 5.3% represents an increase of about one-third over the low producer.
3. In 1940-41 we secured two clippings in each of the five months December to April, inclusive, and one clipping in May.
4. In 1940-41 the low-yielding variety Richland produced 9,135 pounds of green weight, while the high producer Nortex×Victoria M 19-17 produced 19,995 pounds, or well over twice as much green matter.
5. In December 1940 and January 1941, arbitrarily called winter, the low producer was Nortex×Victoria M 19-34 with 2,096 pounds. The high producer was Nortex×Victoria M 19-17 with 6,146 pounds. Here we have two selections from the same parental cross, but one yielded about three times as much winter green feed as did the other.
6. In February and March 1941 arbitrarily called early spring, the low producer was Markton C. I. 2053 with 2,129 pounds. The high producer was Nortex×Victoria M 19-17, which also led in winter yields, with 10,125 pounds. Here the highest was nearly five times as productive in green material as was the lowest.

7. In April and May 1941, arbitrarily called late spring, the low producer was Richland C. I. 787 with 2,410 pounds. The highest was Nortex X Victoria M 19-34 with 5,767 pounds, or a little more than twice as much as the lowest.
8. Taking the three top producers of each of the three arbitrary seasonal divisions, we found that of the nine seasonal leaders, seven had Victoria parentage. Nevertheless, other strains with Victoria parentage were sometimes the lowest producers.

The two years results reported here are too preliminary to warrant any more detailed publication. However, the progress report on this work was given at the annual meeting of the Society in Washington in November 1941 and this note is published in the hope of focusing more attention on this new approach to the problems of winter pasture from the small grains.

More detailed figures will be made available to any interested investigators upon request to the author.—IDE P. TROTTER, *Department of Agronomy, Agricultural and Mechanical College of Texas, College Station, Tex.*

## AGRONOMIC AFFAIRS

### CONSERVATION OF SCHOLARLY JOURNALS

THE AMERICAN LIBRARY ASSOCIATION created this last year the Committee on Aid to Libraries in War Areas, headed by John R. Russell, the Librarian of the University of Rochester. The Committee is faced with numerous serious problems and hopes that American scholars and scientists will be of considerable aid in the solution of one of these problems.

One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical periodicals. The attempt to avoid a duplication of that situation is now the concern of the Committee.

Many sets of journals will be broken by the financial inability of the institutions to renew subscriptions. As far as possible they will be completed from a stock of periodicals being purchased by the Committee. Many more will have been broken through mail difficulties and loss of shipments, while still other sets will have disappeared in the destruction of libraries. The size of the eventual demand is impossible to estimate, but requests received by the Committee already give evidence that it will be enormous.

With an imminent paper shortage attempts are being made to collect old periodicals for pulp. Fearing this possible reduction in the already limited supply of scholarly and scientific journals, the Committee hopes to enlist the cooperation of subscribers to this JOURNAL in preventing the sacrifice of this type of material to the pulp demand. It is scarcely necessary to mention the appreciation of foreign institutions and scholars for this activity.

Questions concerning the project or concerning the value of particular periodicals to the project should be directed to Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, N. Y.

### SUMMER MEETING OF CORN BELT SECTION POSTPONED

THE SUMMER MEETING of the Corn Belt Section of the Society for 1942 which was scheduled to meet at the Kansas Agricultural Experiment Station has been postponed.

After contacting the heads of the departments of agronomy in the states of the Corn Belt Section, it has been decided that it would not be wise to attempt to hold the meetings this year because of the automobile and tire situation and the demands on the time of agronomists in connection with the war program.—R. I. THROCKMORTON.

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## NEWS ITEMS

DR. LAWRENCE C. WHEATING of the State College of Washington was called to active duty with the Army on February 11. Doctor Wheating was President of the Western Society of Soil Science, and those intending to present papers at the meeting of the Society in Salt Lake City in June are now requested to communicate with Dr. T. L. Martin, Brigham Young University, Provo, Utah, Vice President of the Society, or with Dr. W. P. Martin of the University of Arizona, Tuscon, Ariz., Secretary of the Society.

—A—

DR. THEODORE E. ODLAND, Agronomist at the Rhode Island Agricultural Experiment Station, was called to active military duty in the Chemical Warfare Service on February 23.



MACK DRAKE, Assistant in the Agronomy Department at the Indiana Agricultural Experiment Station, has been called to active military duty.

—A—

NOTICE has been given that the 1942 annual meeting of the Canadian Seed Growers' Association will be held at the Provincial School of Agriculture at Olds, Alberta, on June 16 and 17.

—A—

IN THE MARCH number of SOVIET RUSSIA TODAY, J. W. Pincus has an interesting article on cotton production in the Soviet, including information on recent progress in cotton breeding and in production methods. While cotton has been grown in Russia for more than 70 years, it is only recently that efforts to increase production have been pushed, including improved cultural and harvesting methods and the breeding of better varieties which have also greatly extended the cotton-growing area in the Soviet Union. Russia is now reputed to hold third place in world cotton production, being surpassed only by the United States and India.

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## PHOSPHORUS FIXATION AS AFFECTED BY SOIL TEMPERATURE<sup>1</sup>

R. R. ROBINSON<sup>2</sup>

IT has been observed that in the southern states fall applications of phosphate fertilizers appear to give better crop response than spring applications, particularly with crops that require a high level of available phosphorus. Since soil temperatures at and near the surface of the soil are higher in the spring and summer than during the fall and winter and since many chemical reactions very nearly double their velocity for a 10° C rise in temperature, it seemed probable that the higher soil temperatures may have increased the rate of phosphorus fixation.

This problem apparently has received very little consideration. A number of years ago Fraps (2)<sup>3</sup> added dilute phosphate solutions to different soils, agitated them at ½ hour intervals for 5 hours at temperatures of 0°, 31°, and 41° C, and then determined the amount of phosphate remaining in solution. With some soils a decrease in phosphate solubility occurred when the temperature increased, whereas with other soils temperature had very little effect. No study was made of the effect of temperature on phosphate solubility as measured by plant response.

The primary objective of this investigation was to determine the effect of soil temperature on phosphorus fixation as measured by plant growth.

### EXPERIMENTAL PROCEDURE

After a preliminary trial had indicated that soil temperature was a factor in phosphorus fixation, a sample of Dekalb silt loam from an old, run-down pasture was selected for further study. Since the soil was strongly acid, it was limed to pH 6.5 and stored outside for a year before being used in this investigation.

A portion of the soil was fertilized with finely pulverized mono-calcium phosphate at the rate of 50 pounds P<sub>2</sub>O<sub>5</sub> per acre on September 11, 1940. After thor-

<sup>1</sup>Contribution No. 29 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, State College, Pa., in cooperation with the Northeastern States. Received for publication October 3, 1941.

<sup>2</sup>Associate Agronomist. The writer wishes to express his appreciation to Dr. E. A. Hollowell, since it was at his suggestion that this problem was investigated.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 306.

oughly mixing, the soil was potted in 1-gallon pots and incubated at optimum moisture content for 2 months. One-half of the pots were incubated in a refrigerator at a temperature of 3° C, whereas the other half were incubated in a section of the greenhouse where the temperature dropped to 15° to 20° C at night but sometimes reached 45° C during the day. The temperatures during the incubation period are summarized in Table 1. In order to prevent excessive evaporation the pots in the greenhouse were covered during the incubation period. Under these conditions the rate of water loss was about the same as in the refrigerator.

TABLE 1.—*Temperatures at which each soil sample in the greenhouse was incubated and period of incubation at each temperature.\**

Hours of incubation at						Total hours
15°-20° C	20°-25° C	25°-30° C	30°-35° C	35°-40° C	40°-45° C	
150	850	138	108	86	58	1,390

\*The samples in the refrigerator were incubated at 3° C for 1,390 hours.

At the conclusion of the incubation period (November 8, 1940) all pots were taken to a section of the greenhouse where the temperature was maintained at 20° to 25° C, and supplementary Mazda light at an intensity of 100 foot-candles was supplied to increase the daylength to 13 hours. At this time one sample of soil that had received no previous fertilizer or incubation treatment was fertilized at the same rate as the incubated soil and potted in 1-gallon pots in the same manner as the incubated soil. One-half of the pots of each incubation treatment and one-half of the pots that were not incubated were then topdressed with monocalcium phosphate at the rate of 200 pounds of  $P_2O_5$  per acre. This amount was believed to be adequate for optimum growth. Samples of unfertilized soil also were subjected to incubation at high temperature, incubation at low temperature, and no incubation. Ladino clover was planted in all pots to measure by plant response the availability of the phosphorus present, and thus indirectly the fixation that had taken place.

The clover in all pots was clipped to a height of 2 inches on January 20, February 27, and April 2, 1941, and the yields of dry matter determined. The first cutting was analysed for total phosphorus. For this analysis, the samples were ignited at 1,110° F, silica dehydrated by the usual procedure, and the silica-free ash taken up with dilute hydrochloric acid. Aliquots were analysed for phosphorus by the method of Fiske and Subbarow (1), using a KWSZ photometer.

#### EXPERIMENTAL RESULTS

The effect of the incubation treatments on subsequent plant growth is shown in Fig. 1 and Table 2. Where no phosphate was applied and where it was applied at the rate of 50 pounds  $P_2O_5$  per acre, incubation at high temperatures resulted in poor growth and low uptake of phosphorus compared with the growth and uptake of phosphorus from pots subjected to incubation at low temperature or to no incubation. The yield of dry matter and uptake of phosphorus from the unfertilized soil incubated at 3° C averaged slightly higher than from the soil fertilized with 50 pounds  $P_2O_5$  per acre and incubated at

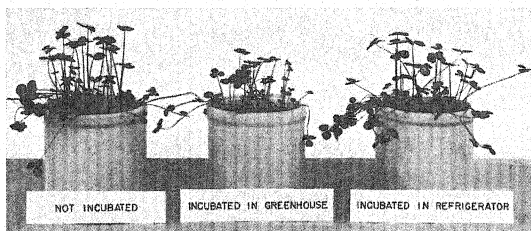


FIG. 1.—The effect of previous incubation treatments on the growth of Ladino clover. The soil not incubated was fertilized at planting time, whereas the others were fertilized prior to the incubation treatment. Fertilization was at the rate of 50 pounds of  $P_2O_5$  per acre. The temperature of incubation is given in Table 1. Photograph taken January 17, 1941, 3 days before the first clipping.

15° to 45° C. A statistical analysis (3) of the data indicated that the differences in yield due to incubation treatments were highly significant except where additional phosphorus was applied at the conclusion of the incubation period (Table 3). Moreover, the differences in the uptake of total phosphorus were also highly significant. It is interesting that in treatment No. 7 (Table 2), in which the soil was incubated at high temperatures but was dry during most of the incubation period, the yields were almost as high as where no incubation treatment was given. Thus, high temperature was an important factor in decreasing the yield only when the soil was moist.

TABLE 2.—The effect of incubating the soil at different temperatures on phosphorus availability as measured by the yield and the phosphorus content of Ladino clover.\*

Treatment No.	Temperature of incubation, °C	Lbs. $P_2O_5$ per acre applied		Yields of dry clover in first cutting, grams per pot	Total phosphorus removed in first cutting, mg P per pot
		Before incubation	At planting		
1	3°	None	None	0.56	1.16
2	15°-45°	None	None	0.11	0.20
3	Not incubated	None	None	0.46	0.90
4	3°	50	None	1.38	2.93
5	15°-45°	50	None	0.41	0.99
6	Not incubated	None	50	1.18	2.64
7	15°-45°†	50	None	1.14	2.24
8	3°	50	200	5.11	16.40
9	15°-45°	50	200	5.41	17.10
10	Not incubated	None	250‡	5.00	15.10

\*All treatments were in quadruplicate.

†This treatment differed from No. 5 in that the soil was occasionally watered to optimum moisture content but was allowed to become dry between watering periods and was dry for most of the time.

‡Fifty pounds were mixed with the soil and then 200 pounds applied as a topdressing.

TABLE 3.—*Analysis of variance of the effect of soil incubation treatments on the yields of dry matter in the first cutting.*

Treat- ments analyzed*	Source of variation					
	Incubation treat- ments		Replications		Error	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square
1, 2, 3, . . . . .	2	0.220†	3	0.014	6	0.014
4, 5, 6, 7, . . . .	3	0.725†	3	0.086	9	0.070
8, 9, 10, . . . . .	2	0.181	3	0.272	6	0.239

\*See Table 2 for explanation of treatments.

†Highly significant; odds greater than 99:1.

The yields with treatments 8 to 10 show that where additional phosphorus was applied at the conclusion of the incubation period, no decrease in yield resulted from incubation at high temperature. It is apparent, therefore, that the adverse effect of incubation at high temperature was due to a high degree of phosphorus fixation.

The calculated percentage phosphorus recovery as affected by the different incubation treatments is given in Table 4. The percentage of phosphorus recovered from soil incubated at high temperature was only 0.2 as compared with 4.3 from soil incubated at low temperature and 3.7 from soil fertilized at planting time. The phosphorus recovered from the soil incubated at high temperature but at a low moisture content was 2.8%. Thus, it appears that practically all of the phosphorus added in the fertilizer was made unavailable to the clover by incubation at high temperatures with optimum moisture.

TABLE 4.—*The effect of incubating the soil at different temperatures on the subsequent recovery of phosphorus in the first cutting of Ladino clover.*

Treat- ment No.	Temperature of incubation, °C	Phosphorus applied, mg P per pot	Total P in herbage, mg per pot	Increase over the check, mg P per pot	Recovery of added phosphorus, %
3	Not incubated	None	0.90	—	—
4	3°	47.3	2.93	2.03	4.3
5	15°-45°	47.3	0.99	0.09	0.2
6	Not incubated	47.3	2.64	1.74	3.7
7	15°-45°*	47.3	2.24	1.34	2.8

\*This treatment differed from No. 5 in that the soil was occasionally watered to optimum moisture content but was allowed to become dry between watering periods and was dry for most of the time.

Only the results of the first cutting, which was made on January 20, 1941, are summarized in Tables 2, 3, and 4. In the second and third cuttings, made on February 27 and April 2, respectively, the results were in agreement with those from the first clipping in that the yields averaged lower where the soil had been incubated at high

temperatures than where it had been incubated at low temperature or where the phosphate was applied at planting time, although a statistical analysis of the data indicated that the differences in dry weight of clippings were of doubtful significance. This general agreement between the successive clippings indicates that the low yields in the first cutting from the soil incubated at high temperature were not due to a temporary tie-up of phosphorus in an organic form as a result of increased microbiological activity. Moreover, in preliminary trials on a mixture of Hagerstown subsoil and sand, where little microbiological activity would be expected, incubation at 20° to 45° resulted in significantly lower yields than incubation at 3° C.

Soil samples taken at the conclusion of the incubation period showed very little difference in available phosphorus as determined by the Truog method (4), using a KWSZ photometer. The soil fertilized at the rate of 50 pounds  $P_2O_5$  per acre averaged 14 pounds per acre of available phosphorus for samples incubated at high temperatures and 16 pounds for those incubated at low temperatures. The untreated soil averaged 9 pounds per acre.

The soluble salt concentration increased in the soil incubated at high temperatures, but apparently this was not the cause of the reduced growth from soil treated in that manner since high yields were obtained where additional phosphorus was applied after the incubation period. However, for a further check on the possible effect of the soluble salt concentration on the yields, a number of extra pots from a previous experiment were still available. These pots had been fertilized and incubated the same as in the present investigation and then kept moist in the greenhouse at 20° to 25° C for 3 months. At the end of this period one-half of the pots were leached until about 2,200 cc of leachate had been collected. The amount of phosphorus removed in the leachate was negligible, averaging 0.4 mg per pot for the soil incubated at 15° to 45° C and 0.6 mg for the soil incubated at 3° C. Part of the pots were then top-dressed with mono-calcium phosphate at the rate of 200 pounds  $P_2O_5$  per acre and Ladino clover planted in all pots.

The dry weights of the clippings from these pots showed that leaching had no significant effect on the yields (Tables 5 and 6). It is interesting to note that the effect of the incubation treatments was

TABLE 5.—*The yields of Ladino clover as affected by the temperature of incubation of the soil and by the accumulation of soluble salts.\**

Temperature of incubation, °C	Lbs. $P_2O_5$ applied per acre		Yields of dry matter, grams per pot	
	Before incubation	At planting	Leached	Not leached
3°	50	None	3.95	3.62
15°-45°	50	None	2.69	2.83
3°	None	200	10.83	9.99
15°-45°	None	200	11.02	10.64

\*All treatments were in quadruplicate.

highly significant in spite of the fact that following these treatments the pots were all kept moist in the greenhouse for 3 months before planting the test crop. As was found in the original trial, differences in yield due to incubation treatments were overcome by applying additional phosphorus at planting time.

TABLE 6.—*Analysis of variance of the growth of Ladino clover as affected by the temperature of incubation and by soluble salts.\**

Source of variation	Degrees of freedom	Mean square
Incubation treatments.....	1	4.233†
Leaching.....	1	0.037
Replications.....	3	0.005
Interaction of incubation treatments and leaching	1	0.211
Error.....	9	0.097

\*The soil heavily fertilized at planting time was not included in this analysis.

†Highly significant; odds greater than 99:1.

### SUMMARY AND CONCLUSIONS

Studies on a Dekalb soil showed that incubating the soil at optimum moisture content for 2 months with the temperature fluctuating between 15° and 45° C resulted in very poor plant growth as compared with incubation at a temperature of 3° C, or with no incubation treatment. That the poor growth resulting from incubation at high temperature was due to a decrease in available phosphorus was shown by the fact that where large amounts of phosphorus were applied at the conclusion of the incubation period, this poor growth was not obtained.

These results suggest an explanation for the observation that in the southern states fall applications of phosphorus fertilizers are more effective than spring applications. Moreover, it would seem that the effect of fall as compared with spring applications of phosphorus to pastures should also be given serious consideration in some of the northern states where temperatures in the surface layer of soil may become high enough during the summer to reduce the availability of the phosphorus.

It is also suggested that part of the discrepancies between the results of field and greenhouse studies on phosphate utilization may be attributed to differences in soil temperature which would influence the rate of phosphate fixation.

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## STRUCTURE OF A DUNKIRK SILTY CLAY LOAM IN RELATION TO pF MOISTURE MEASUREMENTS<sup>1</sup>

VERNON C. JAMISON<sup>2</sup>

FOR many years soil scientists have tried to give quantitative expression to structural differences in soils. Dry and wet sieve aggregate analyses have proved helpful, but some soils may be quite similar in aggregate size frequencies and show marked differences in states of packing, and hence in aeration and permeability. Fruitful studies have been made on soil porosity. Since large pores are important in allowing for good aeration and drainage and fine pores in holding stored moisture, methods are used for determining the "non-capillary" and "capillary" porosities, but the pore space in all soils is not separable into two distinct categories. In our laboratory and elsewhere technics have been used that give pore size distribution information.

In this work, undisturbed soil samples were taken from the same soil type. Complete pF moisture relations were plotted from tension, centrifugal, and vapor pressure data. The derived pore size distributions were studied statistically to bring out significant differences related to field treatments and crop yields. Crop responses often seem to be more related to physical variations than to fertility levels of the soils involved. This experiment was planned to give some quantitative expression to such differences and to test the application of pore size distribution methods to the practical study of field soils.

### REVIEW OF LITERATURE

In 1907, Buckingham (6)<sup>3</sup> proposed the term "capillary potential" as an energy expression of soil moisture conditions. In 1920, Gardner (9) defined  $\Psi$ , the capillary potential, as the work done against the capillary field force in moving unit mass of water from the flat water surface to the point in question. He reasoned that each soil moisture constant should be at an equipotential level.

Schofield (18) proposed the term pF in analogy to the pH scale. He defined it as the base 10 logarithm of the soil moisture tension expressed in cm of water.

Haines (10) and Donat (7) designed simple apparatus, modifications of which have been used in our laboratory (4), by Baver (1, 2), and by Leamer and Lutz (12) for obtaining pore size distribution data. Richards (13) and Woodruff (23) have used compressed air in the soil chamber to determine the moisture content at differential pressures above 1 atmosphere. Bouyoucos (3) introduced the freezing point depression method for determining the "wilting point". Schofield and daCosta (19) modified this technic, calculating energy relations by the use of the Clapeyron equation. They established pF 4.2 as an approximate value for the "wilting point".

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 320.



Briggs and McLane (5) first used the centrifuge to study the moisture-holding properties of soils. However, no sound theoretical calculation was used to compute the energy values involved until Thomas and Harris (21) tried to demonstrate the validity of the equation now quite generally accepted. Schaffer, Wallace, and Garwood (17) performed experiments that proved the soundness of this relationship. The theory enabled Gardner (8) to measure the capillary potentials of small samples over a wide moisture range. Russell and Richards (15, 16) adapted this method to a direct study on soil.

Thomas (20) first reported vapor pressure curves for soil and found the vapor pressure at the wilting percentage to be about 1.1% less than that at saturation.

The most complete moisture tension data for a series of soils were those reported by Russell (14, 15). Other tension or pF moisture curves published have been incomplete, derived by indirect methods, or obtained by calculations based on unsound relationships.

### THEORETICAL

To understand the methods used in moisture tension studies, one should have some notion of the rather simple theory involved.

The following general equation refers to any gas-liquid interface:

$$\Delta p = S(1/r_1 + 1/r_2) \quad 1$$

where  $\Delta p$  is the interfacial pressure difference,  $S$  is the surface tension, and  $r_1$  and  $r_2$  are radii of curvature normal to each other at the point in the surface considered. For a simple capillary tube, the following can be assumed:

$$\Delta p = 2S/r \quad 2$$

where  $r$  is the radius of the tube, being positive when the surface is concave to the gas phase.

For a soil column dense enough to maintain continuous water films from a flat water surface to a height  $h$ , taking the density of water at 25° C as unity:

$$hg = -P = \Delta p \quad 3$$

where  $P$  is the hydrostatic pressure and  $g$  the gravitational coefficient. The rate of change of pressure with height is given by:

$$\frac{-dP}{dh} = \frac{d\Delta p}{dh} = g \quad 4$$

There is also a continuous decrease in the vapor density with  $h$ . Under isothermal equilibrium conditions, soil at  $h$  in the column and a sample of the same soil suspended at the same level will have the same moisture percentage. The change in free energy of the soil water would be independent of the path it moved in going from the flat water surface to  $h$ . Since the free energy of mass  $m$  of water is  $\Psi$ , then in C.G.S. units:

$$\Psi M = Mgh = RT \ln p'/p \quad 5$$

$$h = \Psi/g = RT/Mg \ln p'/p \text{ and } \frac{d\Psi}{dh} = g \quad 6$$

where  $M$  is the molecular weight of water,  $R$  the molal gas constant,  $T$  the temperature in degrees Kelvin,  $p'$  the water vapor pressure in

the soil at  $h$ , and  $p$  the water vapor pressure at the flat water surface. Taking the base 10 logarithm:<sup>4</sup>

$$pF = \log h = \log \Psi/g, \text{ etc.} \quad 7$$

For a porous medium in equilibrium in a centrifugal field of sufficient magnitude to neglect the effect of  $g$ , Gardner has shown that:

$$\frac{d\Psi}{dr} = -\omega^2 r \quad 8$$

where  $\omega$  is the angular velocity and  $r$  the distance from the rotational axis to a point in the porous body. When  $\omega$  is constant, the difference in the potentials between two values of  $r$  is given by:

$$\Psi_1 - \Psi_2 = \omega^2/2 (r_2^2 - r_1^2) \quad 9$$

If a free water surface is maintained at  $r_2$ , then the capillary potential at any point  $r_1$  is given by:

$$\Psi = (\omega^2/2) (r_2^2 - r_1^2) \quad 10$$

For experimental purposes the two values of  $r$  are fixed and it is convenient to express the speed of the centrifuge in revolutions per minute. Then  $\Psi = N^2 C$ , where  $N$  is the R.P.M. and  $C$  is a constant for the experimental technique. And where  $\log C = K$ , then

$$pF = 2 \log N + K \quad 11$$

The "effective pore diameter" is the soil pore size corresponding to the diameter of a simple capillary tube capable of holding water against the same tension. If  $D$  is the effective pore diameter in microns, then from equations 2 and 3 we get the following approximate relations:

$$h = 4S/Dg = 3000/D \text{ (at } 25^\circ \text{ C)} \quad 12$$

$$pF = 3.48 - \log D \quad 13$$

It is apparent from the above considerations that over the  $pF$  range experimental methods are concerned with determining  $h$ ,  $\Delta p$ , or  $\Psi$ . These are related to each other, to  $pF$  and to  $D$  in rather simple ways. The frequency graphs of the same set of soil data will have the same form whether expressed on a  $pF$ ,  $\log D$ , or  $\log \Psi$  scale.

## EXPERIMENTAL

The plots used are located on Caldwell Field, at Ithaca, N. Y. They are 1/100 acre units, being 43.6 feet by 10 feet, and are arranged in duplicate north and south sets with field Nos. 5506 to 5516 and 5706 to 5716, respectively. Their history from 1933 to 1938 is shown in Table 1. With one exception, duplicate treatments were used on plots of the same individual number located in opposite positions across a roadway. Plots No. -- 11 were treated alike except for the organic matter added. Duplicate plots No. --- 8 to No. -- 10, inclusive, were plowed from timothy in 1934 and continuously fertilized and cropped. Plots No. --- 6 and No. --- 7 were broken from timothy in 1938. The two series No. -- 15 to No. -- 12 were left unfertilized and down to grass until the spring of 1939. These groups were broken that year and the No. -- 16 plots were plowed from timothy in 1940.

<sup>4</sup>The symbol "ln" shall refer to the base  $e$  and "log" to base 10.

TABLE I.—History of plots from 1931–1938.

Year	Nos. 5516–5512 Nos. 5716–5712	No. 5511 No. 5711	No. 5510 No. 5710	No. 5509 No. 5709	No. 5508 No. 5708	No. 5507 No. 5707	No. 5506 No. 5706
1931	Timothy	Timothy	Timothy	Timothy	Timothy	Timothy	Timothy
1934	Timothy	Timothy 500 lbs. S. P.* 200 lbs. KCl	Oats and peas; sudan grass; rye 2,000 lbs. NaNO <sub>3</sub> 500 lbs. S. P. 200 lbs. KCl	Timothy; fodder corn; rye 2,000 lbs. NaNO <sub>3</sub> 500 lbs. S. P. 200 lbs. KCl	Timothy; buck- wheat; rye 1,000 lbs. NaNO <sub>3</sub> 500 lbs. S. P. 200 lbs. KCl	Timothy 3,000 lbs. NaNO <sub>3</sub> 500 lbs. S. P. 200 lbs. KCl	Timothy 500 lbs. S. P. 200 lbs. KCl
1935	Timothy	Oats and peas; sudan grass; rye; organic matter†	Oats and peas; sudan grass; rye 2,000 lbs. NaNO <sub>3</sub>	Rye; fodder corn; rye 2,000 lbs. NaNO <sub>3</sub>	Rye; buckwheat; rye 1,000 lbs. NaNO <sub>3</sub>	Timothy 3,000 lbs. NaNO <sub>3</sub>	Timothy
1936	Timothy	Oats and peas; sudan grass; rye; organic matter† 2,000 lbs. NaNO <sub>3</sub>	Oats and peas; sudan grass; rye 2,000 lbs. NaNO <sub>3</sub>	Rye; fodder corn; rye 2,000 lbs. NaNO <sub>3</sub>	Rye; buckwheat; rye	Timothy	Timothy
1937	Timothy	Rye	Rye	Rye	Rye	Timothy 3,000 lbs. NaNO <sub>3</sub>	Timothy
1938	Timothy	Flint corn 63 lbs. T. V. A. metaphosphate 125 lbs. NaNO <sub>3</sub> 47.6 lbs. Ura- mon 150 lbs. 58% KCl	Flint corn ferti- lized same as No. 11	Flint corn ferti- lized same as No. 11	Flint corn ferti- lized same as No. 11	Flint corn ferti- lized same as No. 11	Flint corn ferti- lized same as No. 11

\*S. P. = Superphosphate.

†20 tons manure in 1935 and 131 tons manure in 1936 added to No. 5511. 48.9 tons Port Byron Peat in 1935 and 42.8 tons Port Byron Peat in 1936 added to No. 5711.

On May 30, 1940, just before the field was plowed, one structure sample was taken from each end of every plot. Tension data were obtained in the laboratory from which complete pF moisture and pore size distribution curves were plotted. A 10-20-10 mixture of sulfate of ammonia, T. V. A. metaphosphate, and muriate of potash was drilled over the whole area at the rate of 600 pounds per acre. After the seedbed was fitted, sudan grass was drilled at the rate of 20 pounds per acre. A good stand was obtained and with a favorable distribution of rainfall, the growth was fairly consistent with the observed physical conditions over the plots. The field was harvested on August 20. Crop yields were determined from fresh weights and moisture determinations on grass samples taken.

The device used to take soil structure samples is shown in Fig. 1. A steel cylinder was beveled so as to vector the packing forces away from the sample. The inside of the cylinder was cut about 1 mm larger in diameter so a waxed fibre cylindrical container would just fit flush with the inside wall of the cutting end. This was held in place by a heavy flanged steel cap used for driving the cylinder into the soil. The containers were 60 mm long and 85 mm inside diameter. The cores removed from the soil were capped and stored until analyzed in a moist storeroom controlled at about 1° C.

To study the lower pF range, several small desiccators were used. A small hole was ground in the lower compartment of each. In this was placed a 00 size one-hole rubber stopper. Through pieces of rubber and glass tubing, the compartment could be connected with a vessel of water that could be adjusted to any desired level within the limits of the room used. A 75-mm Coors porous clay disc was sealed on the screen rest of the desiccator with DeKotinsky cement. The lower compartment was filled with water and the adsorbed air removed from the disc by attaching the outlet to a reduced pressure line while the vessel was inverted in a pan of water. This device is also shown in Fig. 1.

Each sample was trimmed even with its container, care being taken to disturb the soil surface as little as possible. The sample was placed on the porous disc of a desiccator, the space between the container and desiccator walls was filled with weighed melted paraffin (about 60° C). To prevent drying of the soil by evaporation a petri dish was inverted over the sample and fitted loosely but evenly into the paraffin while it was still soft. The water vessel was connected and the water level adjusted about 5 cm above the top of the soil until it was flooded. The excess water was removed and the desiccator detached and weighed to 0.1 gram. The water vessel was attached again and the level adjusted to about 4 cm below the center of the sample and the system left for 2 days to come to equilibrium. This time interval was shown to be more than adequate for any tension used on the type of soil studied. Weighings were made at levels set so as to give approximate successive tensions of 0, 4, 16, 40, and 160 cm water. The values recorded were measured from the center of the soil sample to the water level with a meter stick.

A pF of about 2.5 was obtained by attaching the desiccator to one of the units of a modified Richard's controlled low pressure device (4) for 2 days. A moisture sample was taken after the last weighing, and from the weights of the desiccator system, the sample carton, and the paraffin, the moisture percentages at each of the tensions were calculated. A correction for removal of water from the porous disc was not necessary. Several preliminary tests showed a loss in weight of less than 0.5 gram for the disc over the entire range used. Samples of the soil were carefully removed from the desiccator and used for vapor pressure and centrifugal studies. The moisture content was also determined on a sample at a tension just short of pF 3 by a differential pressure technic (11).

The trunion cups used for centrifuging were prepared in a manner similar to that described by Gardner (8), the only essential difference being the use of asbestos fibre conducting columns (11). The time found necessary for equilibration at various tensions was much the same as that found by Russell and Richards (16). It was found that the destructive puddling effect could be markedly reduced by bringing cups containing wet soil to the desired speed slowly (11).

The samples saved from each soil for vapor pressure study were placed in open weighing cans over sulfuric acid solutions of known densities in large pyrex desiccators. Moist soils in the desiccator were found to change in weight for more than 2 months when the ordinary evacuation method was used. This equilibration

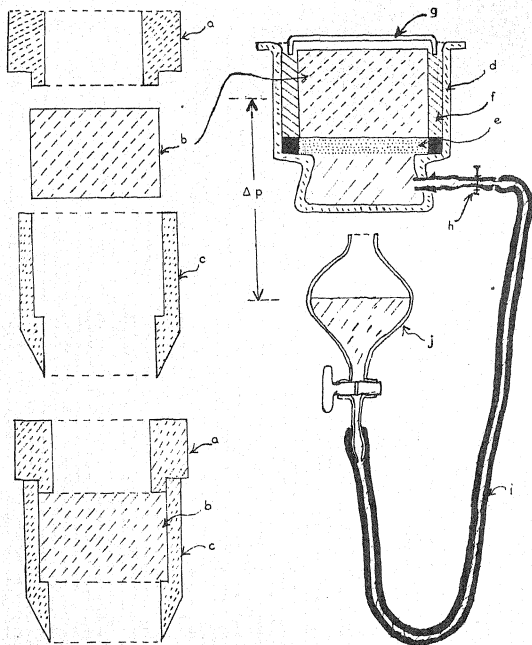


FIG. 1.—Sampling device and low tension apparatus. Vertical cross section of sampler cap (a), soil sample in carton (b), sample cylinder (c), small desiccator (d), porous plate (e), paraffin (f), perti dish cover (g), pinch clamp (h), pressure tubing (i), and separatory funnel (j).

period was reduced to less than 2 weeks by using a stirred chamber method (11).<sup>5</sup> The remaining desiccators were evacuated and all of them kept in the dark at a constant temperature of  $25^{\circ} \pm 0.1^{\circ} \text{C}$  for a month. The samples were removed and the moisture content determined. The density of the acid was determined by use of a set of hydrometers calibrated to 0.001. The data of Wilson (22) were used to obtain the water vapor pressures corresponding to the acid densities. The equilibrium pF values were calculated from the relationship  $\text{pF} = 6.51 + \log (\log 23.756/p)$  obtained from equations 6 and 7.

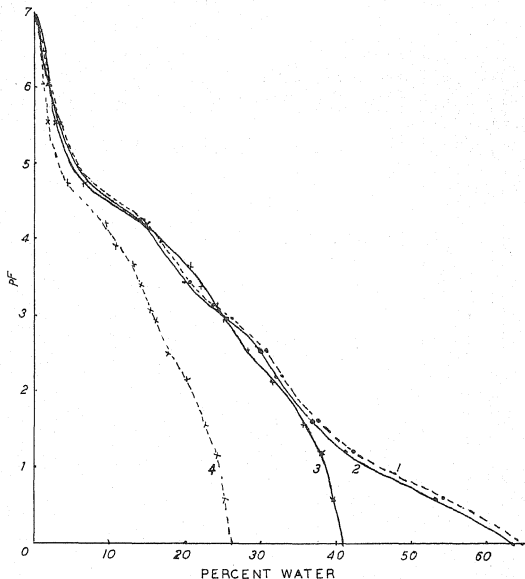


FIG. 2.—Comparison of two means of moisture expression for a surface sample in good structure and a subsurface sample taken nearby. 1, No. 5516, %  $\text{H}_2\text{O}$  of dry weight; 2, No. 5516, volume %  $\text{H}_2\text{O}$ ; 3, subsoil No. 5507, volume %  $\text{H}_2\text{O}$ ; 4, subsoil No. 5507, %  $\text{H}_2\text{O}$  of dry weight.

### RESULTS

The pF moisture data for the different soil samples were combined and the complete pF curves were plotted. In Fig. 2, the curves are shown for a surface and a subsurface sample. Curves 1 and 2 are the

<sup>5</sup>Following a suggestion by Dr. C. M. Woodruff of Missouri.

dry weight and volume percentage moisture tension relationships for a sample taken from plot No. 5516 just before it was broken from timothy. The curves are not very different since the volume weight of this soil was 0.98. However, there is considerable spread in the curves for the subsurface sample used, the volume weight of which was 1.57.

Moisture percentages are conveniently expressed on the dry weight basis, but where one is interested in the volume of pore space of large and of small pores, it seems logical that expression of moisture on the volume basis gives the more nearly true picture. It is true that a clay soil upon drying shrinks somewhat in volume. This objectionable feature of the volume expression may be partly overcome by using the total volume found at saturation as a base.

In Fig. 3 are shown the volume weight curves for all of the samples taken from the north series of second year cultivated plots.<sup>6</sup> The dry weight percentage curves are not shown here, but the differences on that basis over the whole range are great, especially below pF 1.5. Russell (14) used the dry weight basis since he found the spread greater in the range near pF 2.0 if volume percentages were used instead. We found this to be true, but the spread in the data was less between pF 0 and 1.5 and also between pF 3 and 7 if the volume instead of weight percentage values were plotted.

In another location on Caldwell Field a profile study was made. In Fig. 4, the curves are shown for duplicate samples taken less than 2 feet apart and at the 0 to 3 inch and 9 to 12 inch levels. Noticeable differences exist between samples very close together, but the curves are enough alike to demonstrate the reliability of the method. The differences in the curves in Fig. 3 must be mainly the result of inherent soil variability since the area represented received essentially the same field treatment for several years.

From the shape of pF curves and the relation between particle and pore size for soil separates noted previously in our laboratory (4), the curves for the subsurface horizon are similar to what one would expect for a coarse clay having little structure. The effective pore diameter of the pores of greatest frequency would be about 100 millimicrons. This corresponds roughly to a particle size of 7 microns in diameter.

It would be difficult to compare two sets of data such as shown in Fig. 3 without resorting to statistical methods. Curve 1 in Fig. 5 was plotted by taking the mean of moisture percentages at equipotential levels for all the curves shown in Fig. 3. The lengths of the parallel lines represent the computed standard errors of the means. The mean curves for the other treatment groups on the north side of the field are superimposed on the same graph. The mean curve for the first year cultivated plots is not shown since it was similar to curve 1. The changes shown represent roughly the degeneration of soil structure with continuous cultivation. The flex point occurring in curve 1 at about pF 1.6 is noteworthy since Baver (2) attached special significance to this level.

<sup>6</sup>Second year since broken from timothy. "Cultivated" to be used in same sense hereafter.

The pore size distributions of these groups are shown in Fig. 6. The whole pore size scale is divided into five fractions. Any pores that should possibly occur larger than 3 mm are included in the larger group of "macro pores". The vertical scale,  $fD$ , is the percentage of the *total space* (solid+pores) in a fraction divided by the difference in the logarithms of the limiting diameters for that fraction. Hence, the area under the indicated mean in each fraction block is the pore

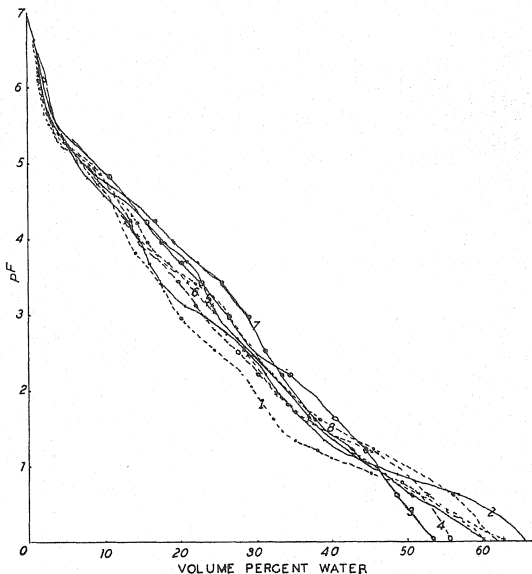


FIG. 3.—The pF moisture curves for samples taken from north series of plots second year continuously cultivated. Samples taken from north and south ends of each plot (symbols N and S). 1, No. 5512N; 2, No. 5512S; 3, No. 5513N; 4, No. 5513S; 5, No. 5514N; 6, No. 5514S; 7, No. 5515N; and 8, No. 5515S.

space percentage that fraction is of the total soil space. The greatest differences between the different treatments are in the macro pores, below  $pF$  1.6. Although the standard errors are large, the only difference between paired treatments in the macro pore fraction that is not significant is second and third year cultivated. The somewhat similar relationships (11) found for the south series groups of plots are not shown here.



On the manured plot, the soil was mellow and friable and failed to form hard clods upon drying as did the adjacent seventh year cultivated plots. However, the pore size frequency failed to express the desirable physical properties shown. The results (not shown here) closely resembled those of the seventh year cultivated series. This was

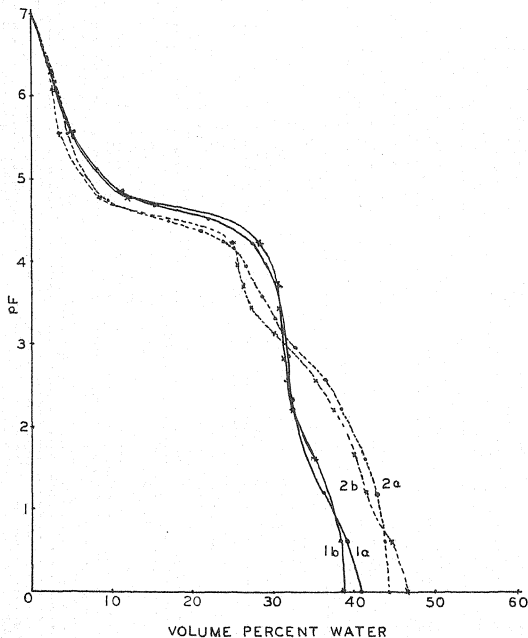


FIG. 4.—Profile study of a Dunkirk silty clay loam. Depths are those of samples. 1a and 1b, B<sub>1</sub> 16-19 inches; 2a and 2b, B<sub>2</sub> 36-39 inches.

not true for the plot to which peat had been added. There was some improvement in structure with a corresponding small but significant increase in yield.

Since there was no snow on the plots and the ground was not frozen on December 25, the first year cultivated plots were sampled again. The mean curves for the samples taken then and in June from these

plots are compared in Fig. 7. The change indicated in the total porosity is highly significant. This has been accompanied by the disappearance of the flex at 1.6. In light of the lack of significant differences between the porosities of these plots and the second year cultivated series in June, it would seem that cyclic changes occur in

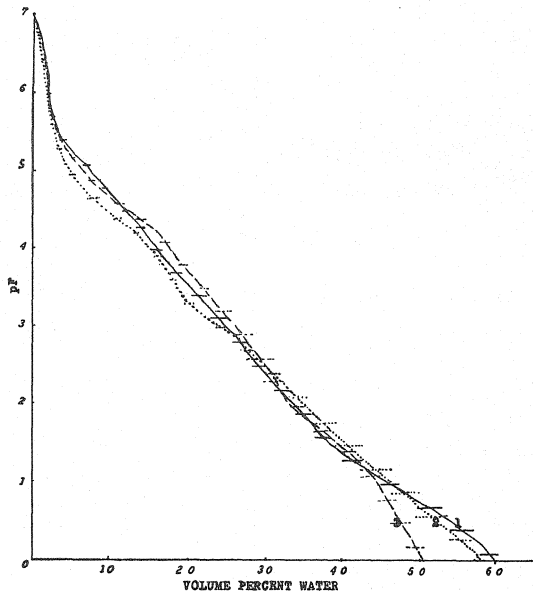


FIG. 5.—The mean pF moisture curves of plot groups in the north series. Standard errors are indicated by lines parallel to base. 1, 2nd year cultivated; 2, 3rd year cultivated; 3, 7th year cultivated.

pore size distribution at the same time there is a general slow degeneration in the structure of the soil with tillage. The former may be due to shifts in the arrangement of the natural aggregates, while the latter is the slow destruction of the aggregates themselves.

The sudan grass yields, calculated to pounds of dry matter per acre, were grouped according to the scheme used in the pore size analyses. Averages and standard errors were computed. The relationship between the macro porosity, pF 0 to 1.6, and yields is shown

graphically in Fig. 8. There is a trend showing an increase in these pores favors productivity. But a similar positive relationship (11) was found for any of the size groups below the wilting point, pF 4.2. For increases above pF 4.2, there was a general negative relationship.

Baver (1) found that the larger pores are positively related to permeability. However, where aeration and moisture supply are also

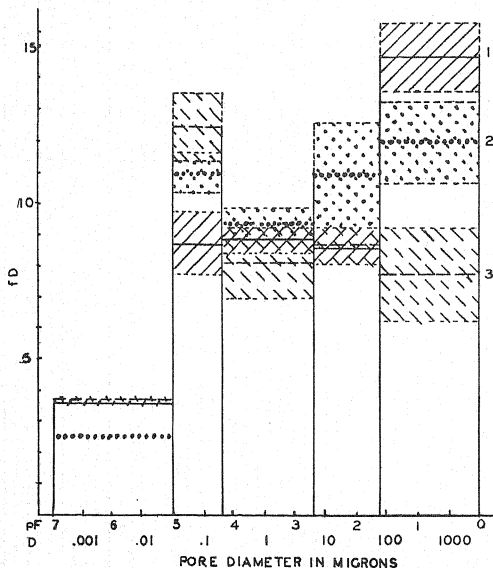


FIG. 6.—Mean pore size distribution for north series of plot groups. Standard errors are shown by shaded areas. 1, 2nd year cultivated; 2, 3rd year cultivated; 3, 7th year cultivated.

factors that limit growth, then pores that are somewhat smaller may also be important.

The failure of the method to indicate the good physical properties of the manured plot must not be overlooked. "Available" potassium may have been a factor affecting growth. The amount of exchangeable potassium on the manured plot, 1.24 M.E. per 100 grams, was about twice that found for any other plot. It is also probable that a state of natural aggregation with a close state of packing existed before plowing when the plots were sampled. With tillage, a desirable

condition for growth was provided for all of the plots, but those with the greatest structure stability would retain the loosening effect longest. It is apparent that supplementary physical measurements would be useful. In addition to following changes in volume weights periodically throughout the growing season, variability in texture should be tested. The use of a quantitative expression of friability

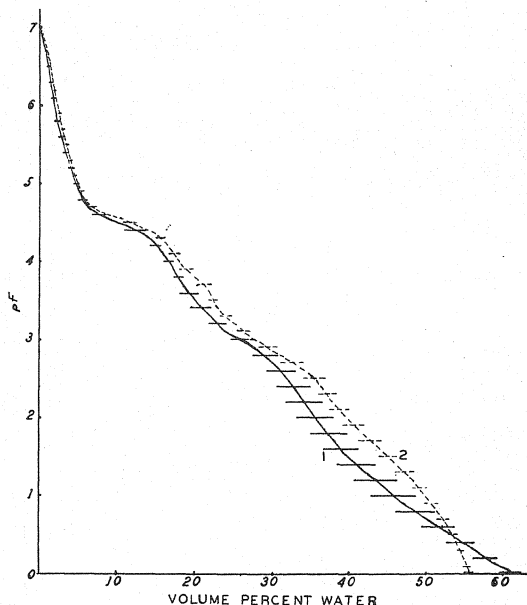


FIG. 7.—Change in 1st year cultivated plots in 6 months time. Plots Nos. 5516 and 5716. 1, sampled June 1, 1940, in sod; 2, sampled December 25, 1940, ploughed and a crop of sudan grass harvested.

would be useful. Perhaps the penetrometer would give valuable information. Some dependable criterion of structure stability would furnish valuable supplementary data.

#### CONCLUSIONS

Volume percentage moisture pF relationships can be used to picture pore size distribution in soils, if the basis of the moisture expression is standardized at saturation.

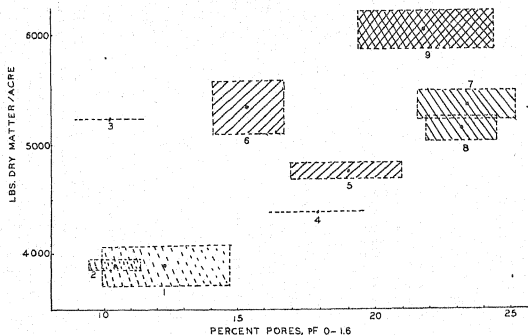


FIG. 8.—Relation between macroporosity and yields of sudan grass in 1940. Shaded areas indicate standard errors. 1, 7th year north series plots; 2, 7th year south series plots; 3, manured plot No. 5511 (only one yield sample taken); 4, peat-soil plot No. 5711 (only one yield sample taken); 5, 3rd year north series plots; 6, 3rd year south series plots; 7, 2nd year north series plots; 8, 2nd year south series plots; 9, 1st year from sod.

For the Dunkirk silty clay loam studied, pF moisture relations show a wide variability over the area of a small plot where the soil appears uniform. This is not due to the method used, since samples taken close together were much more alike than those taken several feet apart. Caution should be exercised in using the calibration curve of a soil sample as a reference for the soil.

A pore size distribution study is helpful in picturing a soil as it is at one time and in one spot. It can be used to follow structural changes if sufficient samples are taken and statistical methods used.

There seem to be two different types of processes causing shifts in the distribution of pore sizes in soil. One is related to the formation or the destruction of natural stable aggregates and the other to factors that effect changes in state of packing. The latter is dynamic in soils under cultivation.

For the Dunkirk silty clay loam, structural improvement as shown by good growth seems to accompany a general increase in the pore space, pF 0 to 4.2.

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## EFFECT OF VITAMIN B<sub>1</sub> ON FIELD CROPS GROWN ON SEVERAL NORTH CAROLINA SOILS<sup>1</sup>

W. W. WOODHOUSE, JR., AND H. D. MORRIS<sup>2</sup>

POPULAR literature has contained during the last two years a number of articles suggesting the use of vitamin B<sub>1</sub> as a growth stimulant for plants. Numerous claims have been made in articles and advertisements which cover a wide range of crops. Commercial fertilizers have been sold at premium prices with their vitamin B<sub>1</sub> content as the selling point. As a consequence, many inquiries have been made, both by fertilizer consumers and manufacturers, concerning the value of vitamin B<sub>1</sub> for increasing the yields of field crops. The purpose of the investigations reported here was to study the effect of vitamin B<sub>1</sub> on field crops.

Bonner and Greene (6)<sup>3</sup> found that vitamin B<sub>1</sub> is synthesized in leaves in light and transported to the root tips. They stated that vitamin B<sub>1</sub> in soils may be derived from plant debris and soil microflora and attributed a part of the beneficial effects of manure to its vitamin B<sub>1</sub> content.

Several workers (1, 2, 3, 4, 5, 9) have shown that vitamin B<sub>1</sub> is necessary for the growth of excised roots of several species, such as peanuts, peas, radish, tomatoes, and flax. Bonner and Greene (7) also reported that the effect of vitamin B<sub>1</sub> is permanent and suggested that the response of a given species to additions of this compound is regulated by the amount of vitamin B<sub>1</sub> synthesized by the leaves of the plant.

West (10) considered the concentration of vitamin B<sub>1</sub> a factor in the increased growth of certain bacteria in the root zone of higher plants.

Galligar (8) studied the correlation between the growth of excised root tips grown in media without vitamin B<sub>1</sub> and the type of food stored in seed, and found that root tips from grains high in starch grew best, while those high in protein grew the least. The growth of roots from those seed which were high in oils and sugars varied widely.

Bonner and Greene (6) reported that concentrations of 1, 0.1, and 0.01 mg of vitamin B<sub>1</sub> per liter applied in sand culture increased shoot and root growth of normally slow-growing species, but fast-growing annuals did not respond to vitamin B<sub>1</sub>.

Practically all reported investigations with vitamin B<sub>1</sub> have dealt with horticultural crops or excised roots grown in synthetic media. Little or no work has been conducted on the addition of vitamin B<sub>1</sub> to fertilizers for field crops.

### METHODS AND RESULTS

Greenhouse pot experiments with three Piedmont and three Coastal Plain soils were conducted to obtain the data reported in this paper. The experimental procedure is summarized in Table 1.

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<sup>2</sup>Assistants in Agronomy.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 326.

TABLE 1.—Summary of experimental procedure.

	Piedmont soils	Coastal Plain soils
Soil type and previous treatment	<i>Cecil clay loam.</i> From field in lespedeza for past 2 years <i>Davidson clay loam.</i> From field in a corn and cotton rotation with heavy fertilization for past 5 years <i>Durham sandy loam.</i> From field in a corn and cotton rotation without fertilization for past 6 years	<i>Norfolk sandy loam.</i> From field in tobacco rotation <i>Coxville very fine sandy loam.</i> From field in peanuts previous year <i>Wickham sandy loam.</i> From field in corn, cotton, and peanut rotation
Vitamin B <sub>1</sub> treatments per pot of 20 pounds of soil	A, Check, no B <sub>1</sub> B, 0.1 mg B <sub>1</sub> applied in fertilizer C, 0.2 mg B <sub>1</sub> applied in water solution D, 0.4 mg B <sub>1</sub> applied in fertilizer E, 0.8 mg B <sub>1</sub> applied in water solution	A, Check, no B <sub>1</sub> B, 0.1 mg B <sub>1</sub> applied in fertilizer C, 0.4 mg B <sub>1</sub> applied in fertilizer
Experimental design	Treatments replicated 5 times on each soil and arranged on Latin squares	Treatments replicated 4 times in pots arranged in randomized blocks; pots in each block rerandomized at 2-week intervals
Crops grown and time grown	Corn, grown 52 days	Corn, grown 45 days Cotton, grown 80 days

The soils were screened and weighed into 2-gallon greenhouse pots. All pots, including checks, were given a uniform fertilization of the equivalent of 1,000 pounds per acre of a 4-8-4 fertilizer, the fertilizer being mixed throughout the soil. Vitamin B<sub>1</sub> treatments applied in the fertilizer were mixed with soil just prior to planting. The treatments in solution were made 10 days later. Plants were thinned to a stand of three per pot.

Height measurements were taken during the growing period and at harvesting. Diameter of stalk was determined at harvesting time for corn. After drying, total dry weights were taken for both corn and cotton and the roots removed from the pots for examination.

Table 2, shows the growth means for corn on the Piedmont soils and Tables 3 and 4 for corn and cotton, respectively, on the Coastal Plain soils.

As a supplement to the above experiments, vitamin B<sub>1</sub> solution was also sprayed on one tobacco plant bed on Durham sandy loam and on one bed on Norfolk sandy loam. Four replications were used on each and no differences in size or vigor could be determined.

#### DISCUSSION

On the Cecil clay loam soil values significantly below the check were obtained with all vitamin B<sub>1</sub> treatments except treatment D.



TABLE 2.—Growth means for corn on Piedmont soils with various vitamin B<sub>1</sub> treatments.

Treatment	Weight, grams	Av. diameter of stalk, 1/32 in.	Average height of stalk, inches		
			At harvest 52 days after planting	42 days after planting	31 days after planting
Cecil Clay Loam					
A, check. ....	41.0	16.4	42.0	36.0	25.9
B, 0.1 mg B <sub>1</sub> in fertilizer	25.4	12.8	41.6	33.6	24.6
C, 0.2 mg B <sub>1</sub> in solution	23.1	12.2	38.6	30.9	23.2
D, 0.4 mg B <sub>1</sub> in fertilizer	34.3	15.6	42.8	34.7	26.4
E, 0.8 mg B <sub>1</sub> in solution	19.6	13.0	40.4	33.0	23.5
Difference between means necessary for significance (1% point)	12.4	2.3	—*	—*	—*
Davidson Clay Loam					
A, check. ....	11.1	11.8	29.4	21.9	14.9
B, 0.1 mg B <sub>1</sub> in fertilizer	10.7	11.6	27.6	21.1	14.6
C, 0.2 mg B <sub>1</sub> in solution	9.9	11.4	26.5	19.9	14.4
D, 0.4 mg B <sub>1</sub> in fertilizer	10.7	11.2	27.7	20.8	14.3
E, 0.8 mg B <sub>1</sub> in solution	9.3	11.2	26.9	19.7	13.7
Difference between means necessary for significance (1% point)	—*	—*	—*	—*	—*
Durham Sandy Loam					
A, check. ....	6.36	11.8	25.6	21.8	14.4
B, 0.1 mg B <sub>1</sub> in fertilizer	9.02	11.6	27.6	21.1	16.6
C, 0.2 mg B <sub>1</sub> in solution	7.64	11.4	27.2	19.9	16.2
D, 0.4 mg B <sub>1</sub> in fertilizer	7.64	11.2	26.4	20.1	15.6
E, 0.8 mg B <sub>1</sub> in solution	7.18	11.2	26.4	19.3	15.6
Difference between means necessary for significance (1% point)	1.38	—*	—*	—*	—*

\*No significant differences.

0.4 mg B<sub>1</sub> in solution. This was true both for weight and for diameter of stalk, but no significant differences were found for height of stalk.

Vitamin B<sub>1</sub> did not produce significant differences on the Davidson clay loam soil for any of the measurements.

On the Durham sandy loam soil treatment B, 0.1 mg vitamin B<sub>1</sub> in fertilizer, produced a significant increase in yield over all other treatments, while treatments C and D produced significant increases over the check. No differences in diameter or height of stalk were obtained.

TABLE 3.—*Growth means for corn on Coastal Plain soils with various vitamin B<sub>1</sub> treatments.\**

Treatment	Av. weight of plants, grams				Av. height of plants, in.			
	Norfolk	Coxville	Wickham	Av. 3 soils	Norfolk	Coxville	Wickham	Av. 3 soils
A, check. ....	4.80	3.75	3.58	4.04	27.5	27.1	27.9	27.5
B, 0.1 mg B <sub>1</sub> per pot	4.35	3.65	3.45	3.82	26.7	28.1	28.4	27.7
C, 0.4 mg B <sub>1</sub> per pot	4.75	4.00	3.92	4.22	26.3	27.2	29.1	27.5

Treatment	Av. diameter of stalks, 1/32 in.			
	Norfolk	Coxville	Wickham	Av. 3 soils
A, check. ....	14.8	13.5	13.2	13.8
B, 0.1 mg B <sub>1</sub> per pot	15.0	13.5	12.7	13.7
C, 0.4 mg B <sub>1</sub> per pot	16.0	14.5	12.5	14.3

\*No significant differences.

No consistent differences were found in the roots of the plants on any of the three soils. The roots were quite crowded in the pots at the time the stalks were harvested.

On the Coastal Plain soils no significant difference was found between treatments as a whole or on any individual soil with either corn or cotton.

It is evident from the foregoing data that the effect of vitamin B<sub>1</sub> varied widely for the six soils studied, producing an increase on one soil, a decrease on another, and having no effect on the four remaining soils.

The increases resulting from applications of vitamin B<sub>1</sub> were produced on a soil which, because of continuous cropping without fertilization, was very low in general fertility. This soil represents an extreme condition and is not nearly so representative of corn soils in the Piedmont and Coastal Plain as the other five.

An over-stimulation of microfloral activity in the soil by the application of vitamin B<sub>1</sub> seems to be the only possible explanation of the decrease in growth of corn found on the Cecil soil. It seems likely that

TABLE 4.—*Growth means for cotton on Coastal Plain soils with various vitamin B<sub>1</sub> treatments.\**

Treatment	Av. weight of plants, grams				Av. height of plant, in.			
	Norfolk	Coxville	Wickham	Av. 3 soils	Norfolk	Coxville	Wickham	Av. 3 soils
A, check. ....	2.88	3.68	3.62	3.39	6.70	9.80	8.80	8.43
B, 0.1 mg B <sub>1</sub> per pot	1.80	3.25	3.55	2.87	6.12	9.55	8.78	8.15
C, 0.4 mg B <sub>1</sub> per pot	2.85	3.65	3.30	3.27	7.22	9.55	8.83	8.53

\*No significant differences.

the population of microorganisms in the depleted Durham soil was lower than that in the Cecil soil which was taken from a legume sod. No determinations of microfloral activity were made.

### SUMMARY AND CONCLUSIONS

Greenhouse pot experiments were conducted in order to compare different rates and methods of application of vitamin B<sub>1</sub>. Corn was grown on three Piedmont soils and corn and cotton on three Coastal Plain soils. Vitamin B<sub>1</sub> decreased corn yields on one soil, increased yields on one soil, and had no effect on the other four soils.

There is no evidence from these experiments that the addition of B<sub>1</sub> is effective in increasing yields of corn and cotton on any of the six soils studied, except on the extremely poor Durham soil. It would not seem advisable to add vitamin B<sub>1</sub> to corn and cotton fertilizers in this area as such addition could not be expected to increase yields generally and might, under some conditions, cause injury.

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## THE TIME AND RATE OF NUTRIENT ABSORPTION BY FLUE-CURED TOBACCO<sup>1</sup>

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RAPID chemical plant tissue tests have been found useful in studying the time and rate of nutrient absorption by plants, the effect of the absorption of one nutrient on that of another, and in determining the range in concentration of a specific nutrient in which it is not a limiting factor in growth to a particular plant (3, 4, 6, 12).<sup>3</sup> It has also been shown that, as a rule, if a given plant is able to maintain a high concentration of nutrients in its conducting tissue, there can be little doubt that the soil has supplied the nutrient requirements of the plant for those particular elements.

The physiological reactions of the tobacco plant are essentially governed by such factors as rainfall and its distribution, temperature, soil conditions, and the supply of available nutrients present in the soil. In the past, a considerable part of the research carried out in connection with the production of flue-cured tobacco has dealt with such factors as the amounts, ratios, and rates of application of nitrogen, phosphorus, potassium, calcium, magnesium, chlorine, sulfur, and boron. However, few attempts have been made to determine when the tobacco used plant nutrients, and how much of the individual nutrients it used during certain periods of growth.

A study was made in 1939 and 1940 at the Tobacco Experiment Station, Chatham, Va., to determine (a) the effects of different forms of nitrogen on nitrogen absorption; (b) the effect of split applications of nitrogen and of potash, as well as fertilizer placement, on nutrient absorption; (c) the effect of different sources of magnesia on magnesia absorption; and (d) at what stage or stages during plant growth maximum absorption of nutrients occurs. The results of this study are herein reported.

### MATERIALS AND METHODS

Yellow Mammoth tobacco was grown on Granville sandy loam soil. Nine treatments were used in duplicate on 1/40 acre plots. Although there were variations in time of application and in source of nitrogen and magnesium, the total application of fertilizer on each plot was equivalent to 900 pounds of a 3-10-6 fertilizer per acre. This application, in addition to the 27 pounds of nitrogen, 90 pounds of phosphoric acid and 54 pounds of potash, supplied 90 pounds each of calcium (CaO) and sulfur (SO<sub>3</sub>) and 18 pounds each of magnesia (MgO) and chlorine.

Phosphoric acid was derived from 20% superphosphate and potash from the

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 338.

proper proportions of muriate of potash (60% K<sub>2</sub>O) and sulfate of potash to give the required amount of chlorine. Magnesia as magnesium sulfate was supplied in all treatments, except No. 3, which received magnesia from dolomitic limestone; and No. 4, where no magnesia was applied. Sufficient calcic limestone was added to all mixtures to produce neutral fertilizers. Variations in the source and time of application of nitrogen are shown in Table 1. This table also shows the variation in time of application of a portion of the potash and method of application of the fertilizer as well as total yield and value of the tobacco crops.

TABLE 1.—*Effect of various treatments on the yield and value per acre of flue-cured tobacco.*

Plot No.	Sources of nitrogen	Yield per acre, lbs.			Value per acre		
		1939	1940	Av.	1939	1940	Av.
1	Urea $\frac{1}{3}$ , ammonium sulfate $\frac{1}{3}$ , sodium nitrate $\frac{1}{3}$ .....	1,096	1,092	1,094	\$191.34	\$205.20	\$198.27
2	Urea $\frac{1}{2}$ , ammonium sulfate $\frac{1}{2}$	1,117	1,116	1,117	186.35	203.60	194.98
3	Urea $\frac{1}{2}$ , ammonium sulfate $\frac{1}{2}$ , magnesium from dolomitic limestone.....	1,085	1,020	1,053	191.98	184.80	188.39
4	Urea $\frac{1}{2}$ , ammonium sulfate $\frac{1}{2}$ , no magnesium.....	1,102	1,072	1,087	210.51	186.00	198.26
5	Standard fertilizer—organic* nitrogen $\frac{1}{3}$ , ammonium sulfate $\frac{1}{3}$ , sodium nitrate $\frac{1}{3}$ ...	1,092	960	1,026	191.30	179.60	185.45
6	Same as No. 5, except sodium nitrate was applied 21 days after transplanting	1,074	992	1,033	195.72	192.40	194.06
7	Urea $\frac{1}{2}$ , ammonium sulfate $\frac{1}{2}$ , one-half the nitrogen was applied 21 days after transplanting	1,170	872	1,021	206.56	155.20	180.88
8	Same as No. 5, except sodium nitrate and one-half of the potash was applied 21 days after transplanting	1,285	1,080	1,183	216.77	208.40	212.59
9	Urea $\frac{1}{2}$ , ammonium sulfate $\frac{1}{2}$ , fertilizer applied in bands 2 $\frac{1}{2}$ inches to each side and 1 inch below root crown	1,280	1,116	1,198	214.27	185.20	199.74

\*A combination of equal portions of nitrogen from cottonseed meal, fish scrap, and process tankage.

A sample of 142 plants, drawn at random, was taken from the plant bed the day that plants were set on the experimental plots. Field samples of plants, drawn at random from the various treated plots, were taken 21, 35, 49, and 63 days after transplanting. Each of the first two field samples consisted of 10 plants, but only 5 plants were taken at the later samplings, due to the large size of plants. Harvesting was begun each year on the date of the last sampling, and it was more than half completed during the next two weeks; consequently, a fifth sampling was not made.

Sap extractions were made on 5-gram samples of plant tissue, taken from the lower part of the midribs of the middle leaves of the plant, at the time field samples

were obtained. Sap studies were made according to the rapid chemical methods employed by Carolus (4).

The field samples were air dried, and the total amounts of nitrogen, phosphoric acid, calcium, magnesium, and sulfur in them were determined according to the methods of the Association of Official Agricultural Chemists (8). Potash was determined according to a modification of the method proposed by Schueler and Thomas (11). Treat 1 gram of dry plant material with 10 ml of 10% sulfuric acid; burn over open flame to dryness and expel the sulfur trioxide fumes; burn residue in an oven to a gray ash, take up ash in 25 ml of dilute hydrochloric acid (1+9) and neutralize with sodium hydroxide, acidify with four drops of 99% acetic acid, add 10 ml of ethyl alcohol, cool, add 5 ml of 30% sodium cobalti-nitrite solution, allow to stand 24 hours at 5° C; filter, wash precipitate five times with 0.01N nitric acid, place the precipitate in original beaker, add 20 ml of 0.5N sodium hydroxide and 75 to 100 ml of hot water, boil 3 minutes; transfer hot solution to an excess of standard 0.1N potassium permanganate in 25 to 50 ml of water and 5 ml of sulfuric acid; add excess of standard 0.1N oxalic acid and back-titrate with standard 0.1N potassium permanganate (9). Plants taken at the first and second samplings were small and stalks and leaves were combined. Leaves and stalks were handled separately for the last two samplings.

#### WEATHER

The distribution of rainfall was considered optimum for the main growing season in 1939; while in 1940, the distribution was poor. Eleven periods of precipitation of more than 0.30 inch occurred in 1939, and 12 such periods occurred in 1940. There were 26 days in 1939 and 28 days in 1940 on which rainfall occurred. In 1939, rainfall was 2.93, 3.18, 1.30, and 2.62 inches, respectively, and in 1940 it was 3.48, 2.45, 1.25, and 1.41 inches, respectively, for the four growth periods between sampling dates. While there was 1.44 inches more rainfall in 1939 than in 1940 for the 63-day growing season, probably the most important difference was the occurrence of four periods of precipitation of more than 0.50 inch during the third and fourth periods of growth in 1939; while in 1940, only one such period occurred. Also, two periods of 10 and 9 days, respectively, occurred during these growth periods in 1940 on which no rainfall was received.

#### RESULTS

##### YIELD AND VALUE

Marked differences in yield and value per acre, due to various sources and time of application of nitrogen, were obtained (Table 1). Replacement of the slowly available nitrogen of natural organics by urea nitrogen resulted in an increase in yield of salable leaf and in value of the crop (plots 1 and 5). An increase in value per acre was secured when nitrate nitrogen was withheld and used as a side dressing (plots 5 and 6). Delayed application of one-half of the nitrogen, when ammonia and urea forms were used, resulted in a lower yield and value per acre than when it was all applied at transplanting (plots 7 and 2). Significant improvement in both yield and value was obtained when one-half of the potash was withheld and used as a side dressing (plots 6 and 8).

Addition of magnesium either in the form of magnesium sulfate or dolomitic limestone did not increase the yield or value of the tobacco.

This probably indicates that this particular soil was not deficient in magnesium, as is often the case in similar soil types where additions of magnesium are often necessary for high yields and quality. Application of fertilizer in bands at the sides of the plants showed a marked increase in yield and value during the first year, but gave no improvement in yield and a reduction in value during the second year (plots 9 and 2).

#### COMPOSITION OF PLANT SAP

Studies of the concentration of plant nutrients in the sap were made to determine if any of the differences in fertilization were reflected in the composition of the sap. The average content of plant nutrients at different stages of growth are shown in Table 2. The effects of individual fertilizer treatments on the composition of the sap were not very great, and these data will not be reported in detail.

TABLE 2.—*Plant nutrients contained in the sap of green tobacco at various stages of growth, average of nine treatments in duplicate.*

Stages of growth	Parts per million				
	NO <sub>3</sub> N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	CaO	MgO
Transplants:					
1939 .....	425	138	7,500	475	1,950
1940 .....	628	75	6,750	550	650
Average .....	527	107	7,125	513	1,300
21 days after transplanting:					
1939 .....	362	117	7,000	400	1,750
1940 .....	666	101	4,722	197	161
Average .....	514	109	5,861	299	956
35 days after transplanting:					
1939 .....	675	337	6,000	768	1,697
1940 .....	478	85	7,389	797	607
Average .....	577	211	6,695	783	1,152
49 days after transplanting:					
1939 .....	171	230	11,777	1,361	1,022
1940 .....	113	70	11,219	1,078	775
Average .....	142	150	11,498	1,220	899
63 days after transplanting:					
1939 .....	60	46	14,833	1,500	1,177
1940 .....	43	65	7,917	1,117	1,050
Average .....	52	56	11,375	1,309	1,114

The nitrogen content of the sap was relatively high in the early stages of growth and fell to a very low level during the fourth growth period. The phosphoric acid content was decidedly lower than that of nitrogen, but it showed a similar trend, reaching a low level as the tobacco approached maturity. In contrast, the potash and calcium oxide contents definitely increased as the season advanced. The magnesium oxide content did not show a definite trend, one year showing

a definite increase and the other year a decrease, as the season advanced.

Data giving the average nitrate nitrogen content of plants receiving different nitrogen fertilization are summarized in Table 3. The inclusion of nitrate nitrogen in the fertilizer did not increase the nitrate content of the plant during the early stages of growth to any appreciable extent (plots 1 and 2). This is in accord with the findings of Anderson, *et al.* (2), who stated after a 5-year study that, "At least we may conclude that there has been no advantage in adding the starter (nitrate) nitrogen." However, Anderson and his associates worked with cigar tobaccos on soils high in nitrogen content. He explains that, "probably in ordinary practice, there is always a sufficient quantity of nitrate nitrogen in the soil to fill the limited requirements of the small plants early in the season."

TABLE 3.—*Nitrate nitrogen in sap of flue-cured tobacco receiving different nitrogen fertilizer treatments, average for 1939 and 1940.*

Plot No.	Nitrogen treatments	Nitrate nitrogen in p.p.m. at growth intervals of			
		21 days	35 days	49 days	63 days
1	Urea $\frac{1}{2}$ , ammonium sulfate $\frac{1}{2}$ , sodium nitrate $\frac{1}{2}$	591	605	187	53
2	Urea $\frac{1}{2}$ , ammonium sulfate $\frac{1}{2}$	575	614	128	40
5	Natural organics $\frac{1}{2}$ , ammonium sulfate $\frac{1}{2}$ , sodium nitrate $\frac{1}{2}$	479	591	77	50
6	Same as No. 5 but sodium nitrate side dressed 21 days after transplanting	386	490	145	51
7	Same as No. 2 but $\frac{1}{2}$ of the nitrogen side dressed 21 days after transplanting	456	454	99	68

It appears in the case of our experiments, which were carried out on soils known to be very low in nitrogen content, that the explanation probably is that the urea and ammonium nitrogen nitrified rapidly enough to fill adequately the limited nitrate requirements of the small plants during the first three weeks following transplanting. The limited absorption of nitrates during this period may be due to the fact that the plants require several days following transplanting to establish a new root system. The use of slowly available nitrogen in the fertilizer (plots 1 and 5) resulted in a lower nitrate content of the sap. Delayed application of a part of the nitrogen (plots 5 and 6, or 2 and 7) caused a lower nitrate content in the plant sap in the early stages of growth. Delayed application of one-half of the potash gave a decidedly higher potash content in the plant sap during all intervals of growth. Tobacco that received no magnesium had a lower magnesium oxide content in the sap during all periods of growth than that which received magnesium as magnesium sulfate or dolomitic limestone, but these two forms showed no difference in effect of magnesium in the sap.



## CHEMICAL COMPOSITION

The data on chemical composition of the dry tobacco plant, as affected by the individual fertilizer treatments, showed no significant differences; and consequently, will not be reported in detail. Anderson, *et al.* (1) found in fertilizer tests with cigar tobacco that different sources of nitrogen did not materially affect the quantity of total ammonium-nitrogen, nitrate-nitrogen, or nicotine in the leaf, nor the ratios between them. The individual fertilizer treatments did not alter appreciably the percentage of total ash, phosphorus, potassium, calcium, magnesium, soluble silica, or iron in the leaf. However, these workers found also that the use of ammonium sulfate in the fertilizer did increase noticeably the percentages of manganese, sulfur, and, to a less degree, alumina in the leaves of tobacco.

Data giving the average chemical composition of the tobacco grown in our experiments in 1939 and 1940 at different stages of growth are summarized in Table 4. The percentage of the various nutrients contained in the plants varied widely for the different periods of growth for the two years that the study was in progress. Plants grown in 1939 contained a higher percentage of the individual nutrients than those grown in 1940. Therefore, only the averages will be considered in the discussion given below.

The composition of tobacco plants varied widely at different stages of growth. Plants at the time they came from the plant bed contained a higher percentage of phosphoric acid than at any other period of growth. The percentage of nitrogen, phosphoric acid, and potash was very high during the first two intervals of growth, but decreased rapidly as the plants approached maturity. The percentage of calcium and magnesium oxides and sulfur increased rapidly during each of the first three intervals of growth, but during the fourth interval there was a slight decrease.

Tobacco leaves contained a markedly higher percentage of nitrogen, calcium oxide, magnesium oxide, and sulfur than did the stalks during the third and fourth periods of growth, the only periods when the leaves and stalks were handled separately. The percentage of phosphoric acid in the leaves and stalks was approximately the same. Tobacco stalks contained a decidedly higher percentage of potash than did the leaves in the third period, but the reverse was true during the fourth period of growth. Davidson (5) found that the composition of tobacco plants varied at different stages of growth. Seedlings as they came from the plant bed contained the highest percentage of phosphoric acid, potash, magnesium, and sulfur. The percentage of nitrogen reached its maximum at the time of topping. As the age of the plants increased, the percentage of phosphoric acid and potash decreased; whereas, the percentage of calcium oxide and chlorine increased.

## RATE OF GROWTH

A study of the rate of growth (leaves and stalks combined), as affected by the various fertilizer treatments, showed no significant differences, and detailed data will not be reported. However, the

TABLE 4.—*Chemical composition of flue-cured tobacco at successive intervals of growth, average of nine treatments in duplicate.\**

Growth intervals	N, %		P <sub>2</sub> O <sub>5</sub> , %		K <sub>2</sub> O, %	
	Leaves	Stalks	Leaves	Stalks	Leaves	Stalks
Transplants:						
1939.....	4.71	—	1.21	—	6.29	—
1940.....	3.53	—	0.82	—	3.71	—
Av.....	4.12	—	1.02	—	5.00	—
0 to 21 days:						
1939.....	4.58	—	0.87	—	4.35	—
1940.....	4.39	—	0.93	—	4.96	—
Av.....	4.49	—	0.90	—	4.66	—
22 to 35 days:						
1939.....	4.75	—	0.98	—	5.97	—
1940.....	3.65	—	0.79	—	5.32	—
Av.....	4.20	—	0.89	—	5.65	—
36 to 49 days:						
1939.....	3.55	3.18	0.77	0.85	4.32	5.95
1940.....	2.76	2.60	0.70	0.74	4.74	4.81
Av.....	3.16	2.89	0.74	0.80	4.53	5.38
50 to 63 days:						
1939.....	2.76	1.45	0.68	0.62	3.46	3.93
1940.....	2.07	1.80	0.61	0.62	4.39	3.16
Av.....	2.42	1.63	0.65	0.62	3.93	3.55
Growth intervals	CaO, %		MgO, %		S, %	
	Leaves	Stalks	Leaves	Stalks	Leaves	Stalks
Transplants:						
1939.....	3.07	—	0.87	—	1.06	—
1940.....	2.06	—	0.92	—	0.44	—
Av.....	2.57	—	0.90	—	0.75	—
0 to 21 days:						
1939.....	2.69	—	0.69	—	0.68	—
1940.....	2.46	—	0.92	—	0.68	—
Av.....	2.58	—	0.81	—	0.68	—
22 to 35 days:						
1939.....	3.23	—	1.04	—	0.74	—
1940.....	3.07	—	0.96	—	0.73	—
Av.....	3.15	—	1.00	—	0.74	—
36 to 49 days:						
1939.....	4.83	1.08	1.09	0.44	0.98	0.51
1940.....	3.94	1.19	1.11	0.56	0.87	0.53
Av.....	4.39	1.14	1.10	0.50	0.93	0.52
50 to 63 days:						
1939.....	4.13	1.19	0.78	0.48	0.89	0.49
1940.....	4.32	1.27	1.10	0.52	0.80	0.42
Av.....	4.23	1.23	0.94	0.50	0.85	0.46

\*Results reported on oven-dry basis.

averages of all treatments by periods clearly show the slow rate of early growth and the rapid growth during the latter portion of the growing season (Table 5).

TABLE 5.—Rate of growth of flue-cured tobacco by periods following transplanting, average of nine treatments in duplicate.\*

Year	Total growth by periods								Total, lbs.
	0 to 21 days		22 to 35 days		36 to 49 days		50 to 63 days		
	Lbs.	%	Lbs.	%	Lbs.	%	Lbs.	%	
1939.....	30	1.9	270	17.7	754	49.3	475	31.1	1,529
1940.....	46	3.1	263	18.0	415	28.5	734	50.4	1,458
Av.....	38	2.5	267	17.8	585	38.9	605	40.8	1,494

\*Results reported on oven-dry basis.

During the first one-third of a 9-week growing season, only 2.5% of the total growth was obtained, and probably most of this growth was made toward the end of this 3-week period. Some time is required for plants to become established in the field following transplanting. Nearly 80% of the total growth was made during the last 28 days of a 63-day growing season. One-half of the total growth was made during the sixth and seventh weeks following transplanting, in 1939; while in 1940, one-half of the total growth was made during the last two weeks before harvest was begun. This reversal of maximum growth from the third period in 1939 to the fourth period in 1940 was probably due to the distribution of rainfall during the growing season. The distribution of rainfall was considered optimum in 1939; while in 1940, the precipitation for the first part of the growing season was heavy, and, as the season advanced, the distribution of rainfall was poor. Similar results have been reported in studies on Havana seed tobacco by Morgan and Street (10). They found that 4% of the dry weight was produced in the first 30 days after transplanting and 56% in the last 20 days of a 70-day growing season. The rate of growth of flue-cured tobacco increased from 1.81 pounds per acre per day on the 21st day after plants were set in the field to 19.1 pounds on the 35th day, and reached a maximum of 43.2 pounds per acre per day on the 63rd day (Fig. 1).

#### RATE OF PLANT NUTRIENT ABSORPTION

No significant differences were found in the rate of absorption of nutrients as a result of the various fertilizer treatments. The average amounts of the different nutrients and the percentage of the total absorbed by tobacco for all treatments during the four intervals of growth are shown in Table 6. Only small amounts of nutrients were absorbed during the first 3 weeks of the growing season. Greater quantities of nitrogen and potash were taken up during the first period of growth than of the other nutrients. Decidedly larger amounts of

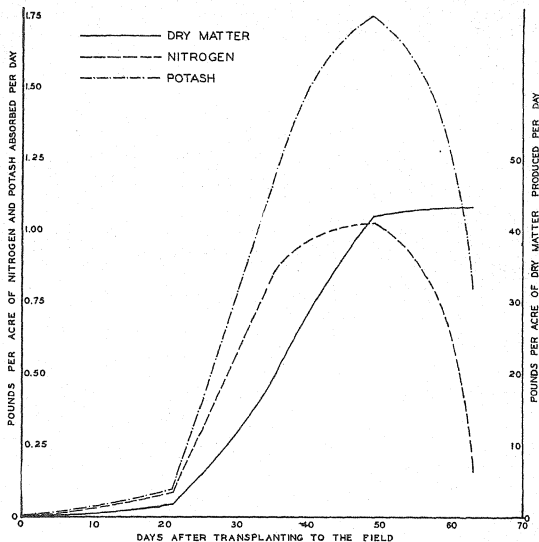


FIG. 1.—Growth and rate of absorption of nitrogen and potash by flue-cured tobacco.

potash were absorbed during the second interval of growth than of other nutrients. However, large quantities of nitrogen and calcium oxide were utilized in this period, also. During the third interval of growth, the absorption of potash was very high, and this was followed closely by the absorption of calcium oxide and nitrogen. More calcium oxide than potash was absorbed during the fourth interval of growth.

Small amounts of all nutrients, except nitrogen, were absorbed and held by the plants during the last 2 weeks before harvest was begun in 1939. It appears that no nitrogen was absorbed after the 49th day in 1939. In fact, a small loss of nitrogen occurred, 3.87 pounds per acre, during this last period of growth. This may have been due to a rainy period which occurred during the latter part of this interval of growth, ending 1 day before field samples were obtained. In 1940, large amounts of nutrients were absorbed during the fourth period of growth. Garner (7) emphasized the importance of rainfall and its distribution in the absorption and utilization of nutrients by tobacco and considered it to be one of the most important variables encountered in the production of tobacco.

TABLE 6.—Quantity and percentage of total nutrients absorbed by flue-cured tobacco at successive growth intervals, average of nine treatments in duplicate.\*

Nutrients	Nutrients absorbed at growth intervals								Total nutrients, lbs.
	1		2		3		4		
	0 to 21 days		22 to 35 days		36 to 49 days		50 to 63 days		
	Lbs.	%	Lbs.	%	Lbs.	%	Lbs.	%	
Nitrogen:									
1939 .....	1.36	—	14.37	—	19.74	—	3.87	—	31.60
1940 .....	2.11	—	9.13	—	8.33	—	8.71	—	28.28
Av. ....	1.73	5.8	11.75	39.2	14.04	46.9	2.42	8.1	29.94
Phosphoric acid:									
1939 .....	0.26	—	2.99	—	5.01	—	0.96	—	9.22
1940 .....	0.44	—	1.99	—	2.74	—	3.83	—	9.00
Av. ....	0.35	3.8	2.49	27.3	3.88	42.6	2.40	26.3	9.11
Potash:									
1939 .....	1.29	—	18.48	—	30.77	—	0.60	—	51.14
1940 .....	2.46	—	14.15	—	17.91	—	21.59	—	56.11
Av. ....	1.88	3.5	16.32	30.4	24.39	45.5	11.10	20.6	53.63
Calcium oxide:									
1939 .....	0.80	—	9.90	—	26.07	—	5.17	—	41.94
1940 .....	1.25	—	8.32	—	12.29	—	22.20	—	44.06
Av. ....	1.03	2.4	9.11	21.2	19.18	44.6	13.69	31.8	43.00
Magnesium oxide:									
1939 .....	0.21	—	3.23	—	5.55	—	0.32	—	9.31
1940 .....	0.43	—	2.55	—	3.75	—	5.71	—	12.44
Av. ....	0.32	2.9	2.89	26.6	4.65	42.7	3.02	27.8	10.88
Sulfur:									
1939 .....	0.20	—	2.25	—	4.50	—	2.11	—	9.06
1940 .....	0.34	—	1.98	—	3.22	—	3.76	—	9.30
Av. ....	0.27	2.9	2.12	23.1	3.86	42.0	2.94	32.0	9.18

\*Results reported on oven-dry basis.

Only a small proportion of the total of each of the different nutrients was absorbed during the first interval of growth. A larger proportion of the total nitrogen was absorbed during the first two periods than of any of the other nutrients. In the third period, the one of greatest absorption, about the same proportion of all six nutrients, 42 to 47% of the totals, was absorbed. Nitrogen absorption was relatively low during the last period of growth.

The rate of growth of flue-cured tobacco and the rate of absorption of nitrogen and potash are shown graphically in Fig. 1. The absorption of nitrogen increased from 0.08 pound per acre per day when the plants were 21 days old to 0.84 pound on the 35th day and reached a maximum of 1.02 pounds per acre per day on the 49th day. Absorption of nitrogen then declined to 0.17 pound per acre per day on the

63rd day. The absorption of potash increased from 0.09 pound per day on the 21st day after plants were set in the field to 1.17 pounds per day on the 35th day and reached a maximum of 1.74 pounds per acre per day on the 49th day. Absorption of potash then declined to 0.79 pounds per day on the 63rd day. A comparison of the rate of growth with the absorption of nutrients indicates that absorption of nitrogen and potash proceeded at a more rapid rate than growth when the plants were young. Maximum absorption of nitrogen and potash occurred approximately 7 to 10 days earlier than maximum growth.

It may be recalled that 27 pounds of nitrogen, 90 pounds of phosphoric acid, 54 pounds of potash, 90 pounds each of calcium (CaO) and sulfur (SO<sub>3</sub>), and 18 pounds per acre each of magnesia (MgO) and chlorine were applied in the fertilizer. Reference to total nutrients absorbed (Table 6) reveals that the tobacco absorbed slightly more nitrogen and the same amount of potash as were applied in the fertilizer. However, the crop removed only about one-tenth as much phosphoric acid and sulfur, nearly one-half as much calcium, and two-thirds as much magnesium as were applied in the fertilizer.

#### DISCUSSION

The slow rate of absorption of nutrients and development of flue-cured tobacco during early stages of growth as contrasted with the very rapid absorption of nutrients and development of the plants during the third and fourth periods were clearly shown. This suggests that only small quantities of plant nutrients need to be available during early growth and emphasizes the importance of maintaining an adequate supply of available nutrients during the middle and latter part of the growing season. The absorption of nitrogen was to some extent an exception in that a large proportion of the total was absorbed in the second period and a very small proportion during the fourth period. A low intake of nitrogen during the fourth period is desirable in order to secure improved quality. Since large quantities of nitrogen are absorbed during the middle of the growing season and high absorption of the other nutrients, except nitrogen, near maturity may be desirable, fertilization procedures should be adopted that will encourage these conditions.

Inclusion of nitrate nitrogen in the fertilizer at the time of transplanting did not result in an appreciable increase of nitrogen in the plant sap or in early growth. In fact, better results from the standpoint of value per acre were obtained when the nitrate was withheld and used as a side dressing. In contrast, where ammonia and urea forms of nitrogen were used, withholding half of the nitrogen to be used as side dressing resulted in a low value per acre. Inclusion of slowly available (organics) nitrogen in the fertilizer resulted in a lower absorption of plant food throughout the season and did not increase the yield or value per acre. It thus appears that forms of nitrogen that are readily available, yet rather resistant to leaching, such as ammonia and urea, are best suited to be used in fertilizer at planting to supply flue-cured tobacco with nitrogen as needed. Results from delayed application of one-half of the potash indicate an



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## IMPORTANCE OF TERMITES IN MODIFYING CERTAIN THAILAND SOILS<sup>1</sup>

ROBERT L. PENDLETON<sup>2</sup>

IN many parts of Thailand outside the low, flat, and intermittently flooded Bangkok plain, termite mounds and the characteristic sorts of trees and shrubs which they carry are a striking feature of the landscape (Fig. 1). These mounds, often 2 to 3 meters high and from 5 to 7 meters in diameter at the base, are hard and difficult to dig into for they are built of relatively heavy textured soil material brought up from some distance below the soil surface. In the process of building these mounds the termites puddle the soil well. The mounds are also striking because of the different uses to which the farmers put the termite mound surfaces as contrasted with the utilization, or absence of it, of the normal soils upon which the mounds stand. This, at once attracts the attention of anyone interested in land use problems. These land use differences are particularly conspicuous on the very poor, acid, sandy laterite soils, and in the padi (lowland, flooded rice) regions (3).<sup>3</sup>

Probably because of the considerable labor involved, Thai farmers are not usually interested in levelling down or obliterating the mounds. Many farmers also believe that the mounds are inhabited by spirits, so that they had best not be destroyed. The farmers prefer to clear the brush and trees from the mounds and at the commencement of the rains, plant on the mound such crops which will very seldom thrive on the infertile soil of the open forest or do not grow at all on the wet, usually saturated soil of the plain round about, such as tobacco, chillies, mulberry, papaya, string beans, cotton, etc. (Fig. 2). Usually the caingin method (2) of clearing and planting is followed. Just before the beginning of the rains the dry brush is burned on the place where it was cut; then after the first rains have sufficiently moistened the surface, seeds or cuttings are planted directly on the mounds, without any preliminary cultivation, in very small holes (a few cm wide and deep) dug in the surface. In north-eastern Thailand the upper half of the mound is often dug down, to give an elevated plot for more intensive garden culture.

Near about the farmstead the termite mounds make an excellent base on which to build the straw stack or to place the rice milling mortar and walking-beam pestle. And in the extensive regions of poor sandy soils, which unless irrigated will not often produce any agricultural crop at all, the mounds in the open forests nearer the occasional villages are frequently cleared, planted to various "up-land" crops and fenced against the cattle which pasture in the forests.

For cultivating rice, which is the universal crop in the lowlands, the Thai farmers use but the simplest of cultivation tools, usually drawn by a single water buffalo, or in some districts by a pair of small cattle. Hence, the mounds scattered here and there in the rice fields

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<sup>2</sup>Agronomist.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 344.

(padi) are not a serious obstruction to cultivation (Fig. 1). In recent years, however, with the putting into effect of an extensive modern highway construction program, and the clearing and levelling of land for certain western-style agricultural experiment stations and for a few power-farming projects, many termite mounds have been levelled off completely, or along the edges of highway alignments, portions of mounds have been cut away.



FIG. 1.—The padi (rice) crop is about ready to be harvested in these productive soils. Termite mounds covered with brush and trees are abundant, for this locality is one not subject to deep flooding. Water for the padi comes from a large irrigation system. Nongkae township, Saraburi Province, central Thailand. December 1937.



FIG. 2.—This large termite mound, planted to various "upland" crops such as chillies and tobacco, stands in a forest clearing (caingin) from which a rains crop of upland rice had been harvested. North of the Lôi valley, northeastern Thailand. March 1938.

When soil material from the base of termite mounds was thus exposed, particularly after a heavy rain, in numerous cases the writer noted on the surface a large proportion of small (1 to 2 by 3 to 6 cm) calcium carbonate concretions, strikingly similar to the "kankar" found in the illuvial horizon of the older soils of the upper Ganges and Jamna plain of north India. Although the different sorts of trees growing naturally on the mounds, and the different ways in which the mounds are used by the farmers, might be explained largely by the differences in topography and texture of the mound material, the presence of the calcium carbonate concretions in the base of the mounds, and the amazing differences in the growth of crops on the site of levelled off mounds as contrasted with growth in the normal soils round about, indicate that the termites had in some way markedly altered the materials making up the mound, or at least they had helped to make more available certain plant nutrient substances.

On spots where mounds have been levelled in tobacco fields the tobacco, if it grows at all, is usually very poor, while cotton and certain other crops do excellently on such spots (Fig. 3), particularly



Fig. 3.—On this fine sandy loam, an infertile, strongly acid lixivium soil, Cambodia cotton grows well on the partially levelled termite mound; elsewhere there is practically no growth. Maecho, northern Thailand Agricultural Experiment Station, Chiangmai Province. December 1935.

if the "levelling" has not been complete, and the site of the former mound is still slightly higher and thus better drained than the surrounding field. On the other hand, on some of the relatively better soils, it has been noted that tobacco grows much better on nearly levelled mounds than on the surrounding normal field soil, while in that same locality cassava (*Manihot utilissima*), which is a strong feeder and thrives best on an especially rich soil, has been reported to grow less well on the site of levelled mounds than on the land about.

In an attempt to discover the nature and the magnitude of the differences between termite mound and the normal soils about them, numerous samples of soil have been collected from various places in

in a number of termite mounds in widely scattered parts of Thailand. Profiles of normal field soils, usually within a few 10's of meters of the mounds, were also sampled for comparison. Samples taken across levelled out mounds in tobacco fields have also been studied. Chemical and mechanical analyses of a considerable number of these termite mound and normal field samples have already been reported upon.<sup>4</sup> These sets of samples were also compared by the use of certain "single-value" determinations, such as the sticky point, the rolling out limit, and the use of the Keen-Raczkowski "box experiment".

The detailed results of these laboratory studies, together with photographs and other data, are published elsewhere (4). Some of the more striking differences which these data show may be summarized as follows:

The higher fertility, and consequently the markedly greater agricultural importance, of the mounds appear to be due to the higher plant nutrient content, the higher pH, and the better moisture relationships of the mound soil material, as well as because the upper portions of the mounds are above the water level on the padi land. As might be expected, there are bigger differences between the normal soil and the termite mound soil in those localities where the soils are very light in texture, thoroughly leached, and strongly acid. Obviously, it is in such regions that the mound soils are particularly important. In localities with younger, less thoroughly weathered soils, the differences between normal and termite mound soils are much less marked.

In general, the termite mound soils usually have a higher air-dry moisture content, and when samples which have been passed through a 1-mm sieve are subjected to the Keen-Raczkowski test, the mound soils usually have a greater pore space, absorb more water, and usually have a greater volume expansion. As a whole, the sticky point and the rolling out limit of mound soils are higher, especially in the sandy regions. However, as the sticky point, the rolling out limit, and the non-plastic range vary with the soil type and the particular horizon, no striking differences between the normal and termite soils are apparent.

From the chemical analyses it is evident that there is a wide variation in the percentage of  $\text{CaCO}_3$  in the samples. Without exception there is a distinctly larger quantity of total Ca in the fine earth of the samples from the mounds than in the normal soils nearby. The two samples from the base of termite mounds which contained lime concretions had 33% and 41% of them, respectively, and of these concretions roughly 35% and 18% were  $\text{CaCO}_3$ . These mounds stood upon acid (pH 5 or lower) white lixivium soils with frequently a laterite horizon at a meter or more below the surface.

As to how the termites can possibly bring about the high concentration of  $\text{CaCO}_3$  in the bases of the mounds without also in-

<sup>4</sup>Since our Department of Agriculture and Fisheries does not yet have a suitable laboratory for the study of soils, we are indebted to Dr. Toa Labanukrom, Director-general of the Department of Science, Ministry of Economic Affairs, and to Nai Sangar Sharasuvana, chief of the Division of Agricultural Science, of that Department, for making these many determinations for us.

creasing proportionately the amounts of other elements which make up their food, it is difficult to imagine. Milne's guesses (1) seem as fantastic as our own. The exchangeable bases in the termite mound soils varied from 2 to 10 times as much as in the normal soil nearby.

A logical, though unexpected result of the laboratory work is that wide differences have been shown in the chemical and physical characteristics of the soil material composing different mounds, and particularly between those which stand on different types of soil in different regions. These chemical and physical soil differences are probably the results of (a) differences in the kinds of termites, for it is likely that there are a number of different sorts of these insects which make similar sorts of mounds; (b) differences in the kinds and amounts of soil materials available for the termites to use; (c) differences in the kinds and amounts of food available for the termites; and (d) differences in the length of time that the mounds have been inhabited, and hence differences in the amount of accumulation of certain products.

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## A LYSIMETER FOR ORGANIC SOILS<sup>1</sup>

J. R. NELLER AND W. T. FORSEE, JR.<sup>2</sup>

AN essential features of a lysimeter installation is that the percolate should be obtained from a soil mass under as nearly a natural or field condition as possible. This percolate is collected from drainage tubes which lead to a tunnel constructed next to or beneath the lysimeter. In a hilly terrain the installation is often on a slope thereby permitting the leachate receivers to be at a level below that of the soil mass in the lysimeters.

In an organic soil, however, the proximity of the soil water table makes impractical the conventional method of collecting the percolate. In most cultivated organic soils the water table is held from 18 inches to 3 feet below the surface. There is, therefore, not only the problem of obtaining the leached material but also that of maintaining a ground water level in the lysimeter itself.

Most organic soil areas are nearly flat and the soil fairly porous so that there is little runoff of rain. An installation in organic soils is simplified in that respect over those in mineral soils where provision has to be made for a runoff of some of the rainfall. These and other lysimeter problems peculiar to the Everglades type of organic soils are discussed below.

As pointed out by Kohnke, *et al.* (3),<sup>3</sup> in their excellent survey and discussion of lysimeters, soil for a lysimeter should be disturbed as little as possible and to accomplish this the monolith or undisturbed soil block is the most ideal. Because of the high water table in organic soils, the monolith type is difficult to establish and the filled-in type was adopted. The high porosity of organic soils, as well as the nature of their profiles, makes it probable that the filled-in type of lysimeter is as satisfactory as the undisturbed soil type.

### EVERGLADES ORGANIC SOILS

The organic soils of the Everglades of Florida comprise an area of about 3,000,000 acres which lie in an unbroken expanse extending from Lake Okeechobee southward and southeastward. Near the lake the soil is deepest, averaging about 12 feet. All of the area is underlain with a porous limestone or marl. The presence of the marl substrata makes it unnecessary to lime these soils. The subsoil waters are profoundly affected by the underlying marl (5) and special consideration was given to this fact in the operation of the lysimeters.

Most of the Everglades area is covered with soil which was formed very largely from a reed-like plant called sawgrass (*Cladium effusum*). This sawgrass peat extends outward from the custard apple muck zone near Lake Okeechobee and the layer is thinner the farther the location from the lake. On the Everglades Experiment Station farm where the lysimeters are installed the peat is about 7 feet deep.

<sup>1</sup>Contribution from the Agricultural Experiment Station of the University of Florida. Received for publication November 24, 1941.

<sup>2</sup>Biochemist in Charge and Associate Chemist, respectively, Everglades Experiment Station.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 352.

The region is nearly flat with a slope of about 3 inches to the mile and as a consequence water control on the cultivated areas is maintained by means of low lift pumps whereby the excess water is pumped into the main canals from a series of field drainage ditches and mole lines (4). On most farms the water table is held about 22 inches below the soil surface. The soil is fairly porous even when it has lost much of its fibrous nature as is the case after several years of water control. Accordingly, most of the rainfall percolates into the soil and it is so disposed of in the lysimeters installed here.

#### DESCRIPTION OF THE LYSIMETERS

In the vast amount of literature pertaining to lysimeters none has been found which described an installation in organic soils. There are probably two main reasons for this, one being that the area of organic soils given over to cultivation has been comparatively small and the other that the presence of a water table near the surface makes the conventional lysimeter design impracticable. In the present instance provision for the collection of leachate and for subsurface irrigation was made by installing the lysimeters in a soil-filled structure above ground level. This box is 4 feet high, 9 feet wide, and 18 feet long. Along both sides of it a shed 32 inches wide (Fig. 1) was constructed to house the percolate and irrigation system.

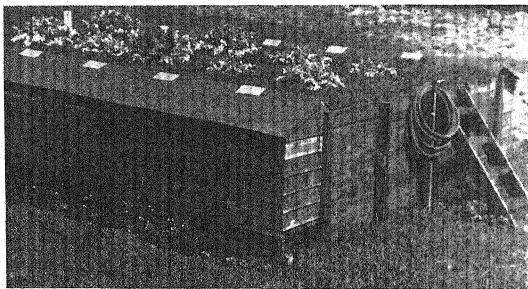


FIG. 1.—General view of a 10 unit lysimeter which provides for a study of a cropped organic soil with a high water table.

The lysimeters consist of 20-gallon glazed crocks with conical bottoms, which were installed in the box on 2 by 6 inch pitch pine stringers, as shown in Fig. 2. The ends of these stringers extend through the walls of the box and are spiked to the 2 by 4 inch up-rights, these being outside the walls of the box. Before the structure was filled with soil all of the wood surfaces were well creosoted. Ten of the 20-gallon jars were thus installed with a minimum distance of 24 inches between jars and outside walls and of 24 inches from jar to

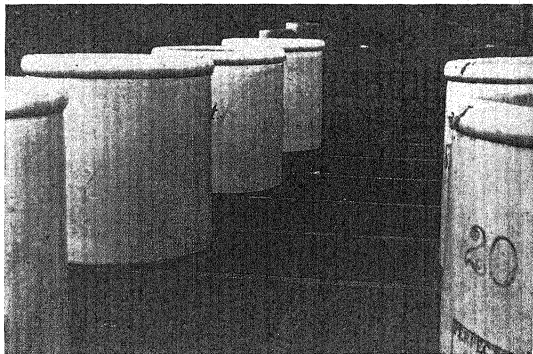


Fig. 2.—Showing the installation of the conical bottomed 20-gallon glazed jar lysimeters. As shown in Fig. 1, the structure was then filled with soil and planted to grass.

jar in a crosswise and 18 inches in a lengthwise direction. Soil was well packed in below and around the lysimeters and the surface was planted to St. Augustine grass. During the two years that the lysimeters have been in operation, the growth of this grass has been similar to that of the grass growing in the field in which the lysimeters are located.

Temperatures in the soil adjacent to the lysimeters have been found to be similar to those in the soil of the lysimeter box (Fig. 3) which indicates that the jars are sufficiently insulated by the surrounding soil. Fig. 3 also shows the prevailing seasonal (A, February 3, and B, October 6) temperatures as well as the diurnal temperature changes.

The outlet of the conical bottom of the lysimeter jar is fitted with a rubber stopper in which the  $\frac{1}{2}$ -inch (inside diameter) block tin tubing is inserted for the conduction of the leachate and of the irrigation water. The rubber stopper and the perforated porcelain plate lying above it are encased in asphalt. The bottom of the jar is set 24 inches above the bottom of the lysimeter box which was high enough to permit the block tin tube to have a slope amounting to a 4-inch drop to the outer wall of the box (Fig. 4A).

To provide for the requirement that the water level in the lysimeters should be maintained by either removing or adding water, the arrangement shown in Fig. 4 is used. This consists of a glass U tube with rubber tubing connection to the block tin tube (A) whereby water enters from bottle (D) when the level falls in cup (B), this being the controlled soil water level in the lysimeter which is an inch



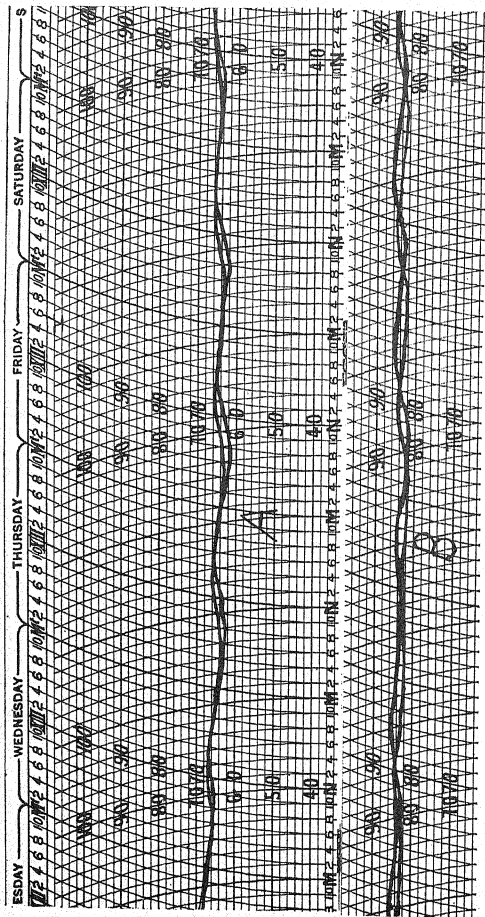


FIG. 3.—Soil temperatures 3 inches below the surface recorded simultaneously in the soil surrounding the lysimeters and in the adjoining field. A, record during the week of February 3, 1941; and B, that of the week of October 6, 1941.

below outlet (C). When, on the other hand, the soil water level gets too high because of rainfall the excess flows out through tube (C) into the leachate receiver. The five outlets along each of two sides of the lysimeter structure are shown under cover in Fig. 1. Five-gallon bottles are used for the collection of the leachate and the inlet tubes are inserted into the mouths of the bottles in cotton plugs to keep out insects. The galvanized squares in the roof of the enclosures (Fig. 1) are removed to permit filling the bottles (Fig. 4D) with water.

#### OPERATION OF THE LYSIMETERS

In November, 1939, a quantity of sawgrass peat was obtained from a field that had been plowed for the first time in 1934. Since the area had been under water control for several years previous to as well as the five years following plowing, the surface soil had lost much of its fibrous nature. In 1934 and 1935 the field had grown crops of forage grasses for which a fertilizer application was used of 200 pounds of sulfate of potash per acre. Copper sulfate at the rate of 50 pounds per acre was also added as it had been previously found by Allison, *et al.* (1) that virgin sawgrass peat lands require copper sulfate to bring them into production.

The top 8 inches of this peat was much less fibrous than that below and it was removed and kept separate from the next 10 inches. These depths were selected as a 20-gallon jar lysimeter provides for an 18-inch soil layer. Below this 18-inch layer the conical bottom of the jar was filled with acid-treated and washed sand. This sand was put through a 16-mesh sieve and the particles that did not pass through were placed over the perforated porcelain disk above the outlet tube upon which the finer fraction was spread. Soil from the lower 10-inch layer was transported to the lysimeters without sieving or breaking up the fibrous mass except as was necessitated in handling it with a shovel. After this layer had been tamped into the lysimeters the upper layer was placed in them. Table 1 records the amount, pH, and moisture content of soil that each lysimeter received.

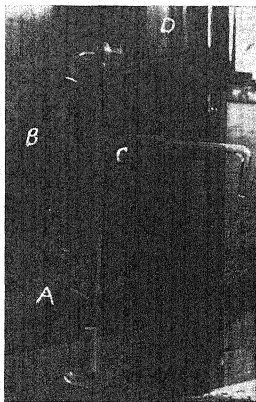


FIG. 4.—Detail of a combined subirrigation and leachate collecting unit. Leachate flows out of drainage tube A and up to level B which is the designated water level in the lysimeter. C is 1 inch above B and soil water rising above B flows out through C to the collecting vessel. When the soil water level falls below B irrigation water flows into lysimeter from D.

TABLE 1.—Data on soil used to fill the lysimeter.

Field sample profile, in.	Reaction pH	Per lysimeter		
		Moist soil, lbs.	Oven-dry basis:	
			Moisture, %	Soil, lbs.
0-8	5.3	59.4	65.94	20.23
9-18	5.5	79.2	81.06	15.00

The side walls of the lysimeter box were marked at B (Fig. 4) to correspond to a water level 17 inches below the soil surface in the lysimeter jars. The water level can be changed by shifting the elevation of cup B. The lysimeters were filled with soil to within 2 inches of their tops so that the rainfall, which often occurs in brief heavy downfalls in this region, would have time to percolate into the soil. As previously mentioned, there is little runoff from Everglades organic soils.

During the dry season which normally extends from October to June much of the water needed by a lysimeter enters from bottle D (Fig. 4). This method of subirrigation is necessary in order to place the soil in the lysimeter jars under as nearly natural conditions as possible. The three gallon Wouff bottles D are filled through the hand holes showing in the roof of the structure (Fig. 1). On sunny days it is necessary to have bottle D full or nearly so each morning, otherwise the heating and expansion of the air in the upper part of the bottle forces water over into the leachate container.

Inasmuch as the soil in the lysimeters is not lying upon the deeper layers of peat which in turn lie upon the marl, it is necessary to use soil water for the subirrigation system. Based upon information previously obtained (5) relative to soil waters, this irrigation water is taken from a shallow well, the collecting reservoir of which is about 2 feet above the marl substrata. Should the lysimeter soil become too alkaline as shown by periodical pH measurements, the irrigation water will be obtained for a time from the Hillsboro drainage and irrigation canal. This water is less hard and the use of it causes saw-grass peat to become more acid in reaction.

During the rainy season which normally extends from June to October rains supply most of the water that the lysimeter soils need with large excess amounts that leach out through the collecting systems. Most of the rainfall passes down into the soil for the reason that, although the rains are generally intense, they do not as a rule continue very long at a time. There are very few rains of 2 inches or more (2). As shown in Table 2, the average annual precipitation is 57.71 inches, most of which occurs during June to September, inclusive.

There is accordingly a considerable amount of leachate from these lysimeters and it is being analyzed for the various plant food elements. These data are being correlated with those of the analyses of crops

TABLE 2.—*Rainfall and evaporation from the open pan from June, 1939, to June, 1941, with averages for previous years at the Everglades Experiment Station.*

Month	Rainfall, inches			Evaporation, inches		
	1940-41	1939-40	Av. 1924-41	1940-41	1939-40	Av. 1924-41
June.....	10.18	11.49	10.00	6.379	6.970	6.233
July.....	4.39	10.30	7.17	6.777	6.617	6.695
Aug.....	8.13	9.78	8.27	6.314	5.256	6.626
Sept.....	8.05	5.60	9.04	4.838	5.763	5.465
Oct.....	2.29	6.59	4.46	5.706	5.090	5.156
Nov.....	0.48	0.15	2.46	4.210	3.936	3.940
Dec.....	6.47	1.46	1.36	2.901	3.314	3.337
Jan.....	4.48	3.34	1.83	3.080	3.163	3.584
Feb.....	5.55	2.72	1.89	3.726	4.423	4.111
March.....	3.17	4.20	3.20	5.498	5.523	5.735
April.....	6.40	1.63	3.52	6.477	6.552	6.619
May.....	3.37	3.00	4.51	8.035	8.077	7.332
Totals.....	62.96	60.26	57.71	63.941	64.684	64.453

grown and removed and of plant food added as fertilizer. As described above, the lysimeters and the leachate collecting systems are constructed of materials that do not release any traces of copper and manganese and probably non-appreciable amounts of zinc and boron. As a consequence, the role and fate of these elements are included in these studies.

Some of the more important crops of the region, such as celery and pasture grasses, are being grown in these lysimeters. For the present a study is being made of one type of soil only, this being the sawgrass peat that comprises so much of the Everglades. It becomes possible, therefore, to include a comparative study of different kinds and amounts of fertilizer treatments of which there are five variations in the experiments now under way.

From a determination of plant food lost to the percolate, information is being obtained relative to losses by leaching under different fertilizer practices. Summation of nutrients leached with that in crop growth removed from the lysimeters leaves a residual of plant food which was in an available form when added, but which is retained or made nonavailable by the soil.

#### SUMMARY

The conventional type of lysimeter with underground outlets is not feasible for a study of arable organic soils for the reason that the water table must be held fairly near the surface in order to have good growing conditions as well as to conserve the soil. Accordingly, the lysimeters described in this paper were installed above the surrounding soil surface in a structure that is filled with soil. Field conditions are thereby maintained except that the lysimeter soil surface is 4 feet above that of the adjacent fields.

Details of the installation are given as well as of a subirrigation device which simulates the type of subirrigation at controlled water table levels as practiced in cultivated lands of the Everglades.

In the present study these lysimeters contain one type of soil only, this being the sawgrass peat typical of most of the Everglades area. Vegetable and grass crops are being grown at different fertilizer levels in the lysimeters and information is being obtained concerning the utilization and availability of added plant food elements, both primary and secondary.

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## GENETICS OF SELF-COMPATIBILITY IN *TRIFOLIUM REPENS*<sup>1</sup>

SANFORD S. ATWOOD<sup>2</sup>

OF fundamental importance to the breeding of any species is a practical means of obtaining inbred lines. With self-compatible plants relatively few difficulties have been encountered in obtaining inbreds, but with species in which the plants have been self-incompatible, the progress of inbreeding generally has been retarded. When attempts were made in white clover to obtain inbred seed by self-pollination, it was found that most plants set very few seeds per head under the conditions tested (7, 5, 2).<sup>3</sup> A few individuals have been found with relatively high pseudo-self-compatibility (3), but several difficulties have been encountered in utilizing this character in a breeding program. Any method, genetic or otherwise, that would assure true self-compatibility would seem to offer considerable promise. Genetic factors for such true self-compatibility have been found in several other species, and there seemed no reason to suspect that similar factors might not be found in white clover. The literature on incompatibility factors in other species has been reviewed recently by Stout (6).

### MATERIALS AND METHODS

The original female parent of the plants used in this investigation was the same plant that was used in a previous study of cross-incompatibility (1) in which the genetic constitution of the plant as regards incompatibility alleles was shown to be  $S_3S_4$ . The male parent came from a seed lot collected in a closely grazed pasture in Preston County, W. Va. These plants were crossed by hand in the greenhouse during the winter of 1938-39, and the  $F_1$  and  $F_2$  generations were planted in the field in 1939 and 1940, respectively. Selections for greenhouse study among both  $F_1$  and  $F_2$  generations were made during their first summer in the field, but the plants were not self-pollinated in the field until their second summer when they flowered profusely. The main bases for selecting among both  $F_1$  and  $F_2$  plants were general vigor, good flowering, and absence of disease, wherever possible. The techniques used for both self- and cross-pollination were the same as those described previously (2).

### EXPERIMENTAL RESULTS

The plant used as female parent was described before (1) as being practically self-incompatible, but during the years of this investigation this same plant showed moderate pseudo-self-compatibility (Table 1). No explanation for such inter-annual variation is available at present, but other evidence has indicated that pseudo-self-com-

<sup>1</sup>Contribution No. 30 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, State College, Pa., in cooperation with the Northeastern States. Also presented at the annual meeting of the Society held in Washington, D. C., November 12 to 14, 1941. Received for publication November 25, 1941.

<sup>2</sup>Associate Agronomist.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited" p. 364.

TABLE I.—Seed set in *F<sub>1</sub>* intercrosses, backcrosses, and selfs.

Genotype*	Plant No.	Seeds per 10 flowers from self- and cross-pollinations in greenhouse												Seeds per head from self-pollination in field							
		9-I (S <sub>1</sub> S <sub>1</sub> )	9-II (S <sub>2</sub> S <sub>2</sub> )	9-III (S <sub>3</sub> S <sub>3</sub> )			9-IV (S <sub>4</sub> S <sub>4</sub> )			9-V (S <sub>5</sub> S <sub>5</sub> )			9-VI (S <sub>6</sub> S <sub>6</sub> )	Av. of comp. crosses	A†	B†	C†				
				9-7	9-8	9-10	9-11	9-14	9-3	9-4	9-6	9-12						9-15	9-16	9-5	9-9
9-I (S <sub>1</sub> S <sub>1</sub> )	9-1	0	46	8†	0†	0†	22	0†	48	25	20	28	0†	45	29	40	43	34.6	16.6	18.6	0.1
9-II (S <sub>2</sub> S <sub>2</sub> )	9-2	47	34	—	—	—	32	34	—	—	(30)§	—	—	—	15	32	52	34.5	159.3	116.6	45.9
9-III (S <sub>3</sub> S <sub>3</sub> )	9-7	11	45	1	0	0	2	0	45	46	45	50	49	36	38	46	50	41.9	7.0	7.7	0.0
	9-8	39	27	0	0	0	1	0	34	29	39	18	28	39	37	38	42	33.6	21.6	16.5	0.8
	9-10	18	27	0	0	0	0	0	29	27	Lost†	28	27	34	32	23	31	27.6	19.2	14.7	1.4
	9-11	(22)	(24)	—	—	—	—	—	(30)	—	—	—	—	—	(36)	(30)	—	28.4	23.6	8.4	0.4
9-IV (S <sub>4</sub> S <sub>4</sub> )	9-14	38	41	0	1	0	1	0	29	44	43	38	12	30	38	35	40	35.3	6.0	4.7	0.3
	9-3	45	38	40	49	38	43	45	0	0	1	1	0	0	47	44	47	43.6	4.9	12.2	0.6
	9-4	37	30	24	23	39	26	31	1	—	0	0	1	0	12	0†	33	28.3	6.2	6.7	0.6
	9-6	14	20	35	40	27	16	23	0	0	0	0	1	0	1	42	29	28.8	1.8	0.7	0.0
9-12	39	44	44	39	36	28	39	0	0	0	0	—	1	0	50	46	50	41.5	15.8	7.5	0.2
9-15	24	43	51	—	50	46	55	0	0	0	0	0	—	2	52	54	35	45.6	5.2	18.2	0.0
9-16	22	44	41	43	34	42	51	0	1	0	0	0	0	2	47	37	34	39.5	3.9	5.4	0.5

	9-5	38	32	38	30	(34)	36	32	32	39	35	38	33	33	48	30	33	34.2	101.4	43.9	10.6	
9-V (S <sub>3</sub> S <sub>1</sub> )	9-9	37	27	41	44	—	43	38	34	40	38	40	39	34	39	36	41	38.2	79.8	72.9	27.9	
9-VI (S <sub>4</sub> S <sub>1</sub> )	9-13	(44)	37	—	—	—	—	50	41	—	—	—	—	—	50	48	37	45.0	94.0	60.5	42.8	
Average of 11 pseudo-self-compatible F <sub>1</sub> plants																			36.2	10.5	9.3	0.4
Average of 3 self-compatible F <sub>1</sub> plants																			37.6	91.7	59.1	27.1

\*The plant numbers and genotype symbols are explained in the text.

†A = Manipulated under bag in 1940; B = manipulated under bag in 1941; C = bagged but not manipulated in 1941.

‡Cross was recorded compatible by reflection of pedicels on third day after pollination. This abnormally low seed-set was omitted from average for plant.

§Numbers in parentheses have been adjusted to a 10-flower basis. For instance, if a pod was lost at harvesting, the total number of seeds obtained was increased by 1/9. In most cases, at least nine flowers were available.

||Plants selected as immediate parents of F<sub>2</sub>.



patibility is heritable (3). The averages of selfed seed resulting from manipulation of entire heads of this plant under bag in the field corresponded closely for the two years (16.6 and 18.6), but as typical of pseudo-self-compatible plants it produced no seed when 10 flowers per head were self-pollinated by hand in the greenhouse (Table 1).

The male parent was originally selected because it was the only plant out of 615 (chosen at random from a nursery of about 10,000) which averaged over 100 seeds per head when self-pollinated under bag in the field in 1938. At that time four heads were enclosed under two bags and the average seed set per head was 143. When clones of this same plant were similarly bagged in 1940 and 1941, average seed sets were 159.3 and 116.6, respectively. As noted in Table 1, this plant was not only self-compatible but also self-pollinating, i.e., autogamous. The average seed set in 1941 when entire heads were enclosed under bags but not manipulated was 45.9. This is less than half the number resulting from manipulation of entire heads, but it is an entirely satisfactory seed set for practical breeding purposes. When additional clones of this plant were selfed by bees under a cage in the field in 1940 (2), the average seed-set of the 75 "best" heads was 173.7, and the average of all 1,567 heads was 113.1. In the greenhouse, this male parent was just as self-compatible as it was cross-compatible. When 10 flowers were emasculated and then selfed with pollen from other flowers on the same plant, 34 seeds were obtained, whereas the average of the seven crosses made with this plant as female was 34.5 seeds per head (Table 1). Likewise, this plant proved self-compatible in the greenhouse when the same selfing technics were applied as were used in the field. In 1939-40, 323 seeds were obtained from a single head that was manipulated several times throughout the duration of flowering, and in 1940-41 a single unmanipulated head set 33 seeds.

It should be evident that this plant showed a high degree of self-compatibility under all conditions that were tested. There have been found (unpublished information) in some other plants of white clover genetic factors for self-compatibility similar to the one to be described here, but very few of these individuals have consistently averaged as many seeds per head as the male parent in this study.

When 14 selected  $F_1$  plants were diallely intercrossed and backcrossed to both parents in the greenhouse, two intra-sterile, infertile groups of five and six plants, respectively, were found, and these 11 plants were reciprocally compatible with both parents (Table 1). Since it had been observed previously that insignificant differences were generally found between different heads used for the same cross, few of the diallel matings were made in duplicate, except where some mechanical damage or disease appeared to be lowering seed-set disproportionately. Some clues to such behavior were secured from noting the degree of pedicel reflection, which had been observed in earlier work to be a good measure of the number of seed that would set. No difficulties were encountered in distinguishing compatible from incompatible crosses. For these 11 pseudo-self-compatible plants, the 106 compatible crosses averaged 36.2 seeds per 10 flowers, while the 46 incompatible crosses, recorded in Table 1, averaged only

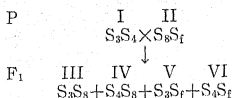
0.33. A similar difference between compatible and incompatible crosses was found in the  $F_2$  generation.

The 11  $F_1$  plants in the two intra-sterile groups were self-incompatible when 10 flowers per head were selfed following emasculation in the greenhouse. When heads were manipulated, however, in either greenhouse or field, considerable seed was obtained. The average number of seeds per head for different plants ranged in 1940 from 1.8 to 23.6, with an average for all plants of 10.5, and in 1941 from 0.7 to 18.2, with an average of 9.3 (Table 1). An insignificant inter-annual correlation ( $r = .4205$ ) was obtained for these 11 pairs of averages. When these same plants were bagged but not manipulated in the field, an average of only 0.4 seed per head was obtained (Table 1).

The other three  $F_1$  plants were cross-compatible with both  $F_1$  groups, with both parents, and with each other. In addition, they were self-compatible like their male parent under all conditions tested. When 10 flowers per head were selfed following emasculation in the greenhouse, they were just as self-compatible as they were cross-compatible, and when manipulated under bag in the field, they averaged 91.7 and 59.1 seeds per head, respectively, for the two years (Table 1). Even when they were bagged but not manipulated, they set an average of 27.1 seeds per head. Differences between these three plants in degree of self-compatibility were of questionable significance. Likewise, there was no significant difference between the cross-compatibility of self-compatible and of self-incompatible individuals in either  $F_1$  or  $F_2$ .

Subsequent to these first crosses, six other  $F_1$  plants were tested in both greenhouse and field. Two belonged to one intra-sterile group, two to the other, and the remaining two were self-compatible. Thus, for the total  $F_1$  there were 15 self-incompatibles and 5 self-compatibles.

The combined results of selfing and crossing with these parents and  $F_1$  plants can be explained best by postulating that the original self-compatible male parent was heterozygous for a self-compatibility factor ( $S_t$ ), which is a member of the multiple-allelic series conditioning self- and cross-incompatibility, and that this factor was transmitted to the self-compatible  $F_1$  plants. This allele differs from the others in the series in that the growth of pollen tubes bearing this factor is not inhibited in any styles, including those bearing  $S_t$ . The second allele contributed by the heterozygous self-compatible male was tested by crossing the two intra-sterile  $F_1$  groups as females with the seven homozygous plants obtained previously (1, 3). As was expected, the pollen from  $S_3S_3$  failed on one of the groups but not on the other, while pollen from  $S_4S_4$  acted in the reverse order. Since all other combinations were compatible, it is evident that the second allele from the male parent is different from the previous seven, and this allele is, therefore, designated  $S_8$  throughout the paper. The following diagram shows the relationship of the various genotypes and the designations used for them throughout the paper:



In order (a) to test the validity of the hypothesis of an  $S_t$  allele and (b) to distinguish the two self-compatible  $F_1$  groups, a progeny test was made as follows: A plant from one of the intrasterile groups was crossed as female with the other group and with the three self-compatible  $F_1$  plants. From the cross between the two groups, the  $F_2$  should consist of equal numbers of the group used as male and the group of the original female parent. Among the three self-compatible plants there could be two genotypes. When one of these genotypes is crossed with the selected group, the  $F_2$  should consist of equal numbers of self-compatible and of self-incompatible individuals; the latter should consist of equal numbers of the group of the original female parent and of the  $F_1$  group not involved in this cross. When the other self-compatible genotype is crossed with the selected group, only self-compatible plants would be expected in the  $F_2$ . Since the  $F_1$  plant selected as female for the  $F_2$  test crosses was shown to bear  $S_4$ , it is so designated throughout the paper:

$$\begin{array}{lcl}
 IV & III & I \quad III \\
 S_tS_8 \times S_3S_8 & \longrightarrow & S_3S_t + S_3S_8 \\
 IV & V & I \quad III \quad VI \quad II \\
 S_4S_8 \times S_3S_t & \longrightarrow & S_3S_4 + S_3S_8 + S_3S_t + S_8S_t \\
 IV & VI & VI \quad II \\
 S_4S_8 \times S_4S_t & \longrightarrow & S_4S_t + S_8S_t
 \end{array}$$

When 48 selected  $F_2$  plants were tested by selfing and by backcrossing to the two parental and four  $F_1$  groups, only the expected types were obtained (Table 2). From the cross 9-IV ( $S_4S_8$ )  $\times$  9-III ( $S_3S_8$ ), all  $F_2$  plants were self-incompatible, and they segregated 8:4 into the two expected groups, 9-I ( $S_3S_4$ ) and 9-III ( $S_3S_8$ ). Two of the self-compatible  $F_1$  plants proved to be of the group 9-V ( $S_3S_t$ ) by the fact that their progeny from the cross with 9-IV ( $S_4S_t$ ) segregated into self-compatibles and self-incompatibles. A ratio of 7 plants of group 9-I ( $S_3S_4$ ):7 of group 9-III ( $S_3S_8$ ):10 self-compatibles was obtained as compared with the expected numbers of 6:6:12. Since a similar deficiency of self-compatibles was found in the  $F_1$  (5 instead of the expected 10 out of 20), this phase of the problem is being investigated further to evaluate its significance. The third self-compatible  $F_1$  plant proved to be of the genotype 9-VI ( $S_4S_t$ ), since all of its  $F_2$  from the cross with 9-IV ( $S_4S_8$ ) were self-compatible.

In addition to the 48  $F_2$  plants selected for greenhouse study, 25 others were grown and selfed in the field. These were classified from their seed-set when bagged both with and without manipulation. All 20 plants from cross 9-IV ( $S_4S_8$ )  $\times$  9-III ( $S_3S_8$ ) proved self-incompatible, those from cross 9-IV ( $S_4S_8$ )  $\times$  9-V ( $S_3S_t$ ) segregated 22 self-incompatible and 14 self-compatible, and all 17 plants from cross 9-IV ( $S_4S_8$ )  $\times$  9-VI ( $S_4S_t$ ) proved self-compatible. In every case the

TABLE 2.—Seed set in  $F_2$  backcrosses and selfs.\*

Cross	$P_2$ geno- types	Number of plants		Seeds per 10 flowers from cross-pollination in greenhouse						Seeds from self-pollination					
		Expected	Obtained	Direction of cross	9-I ( $S_3S_4$ )	9-II ( $S_4S_1$ )	9-III ( $S_3S_8$ )	9-IV ( $S_3S_8$ )	9-V ( $S_3S_1$ )	9-VI ( $S_3S_1$ )	Greenhouse			Field	
9-IV×9-III	9-I	6	8	♀ ♂	0.2 0.1	27.9 43.0	32.6 18.7	31.2 31.3	32.1 21.6	39.5 39.3	0.1	0.4	0.0	1.4	0.1
	9-III	6	4	♀ ♂	29.8 30.7	24.0 41.3	0.2 0.5	31.2 35.3	15.7 27.7	24.2 45.0	0.0	3.2	—	4.2	0.2
9-IV×9-V	9-I	6	7	♀ ♂	0.1 0.1	38.6 49.0	33.3 28.2	33.1 29.0	30.6 29.8	32.6 43.0	0.1	0.3	0.0	3.2	0.4
	9-III	6	7	♀ ♂	42.1 30.3	34.0 40.3	0.3 0.0	31.4 33.8	30.0 36.9	32.6 41.3	0.0	3.6	0.0	7.1	0.8
9-IV×9-VI	9-II or 9-VI	12	10	♀ ♂	33.7 33.0	22.3 39.8	35.4 28.1	32.5 26.6	32.4 26.6	31.1 45.0	18.0	93.4	19.0	36.1	20.6
	9-II or 9-VI	12	12	♀ ♂	37.7 30.9	29.9 43.0	33.6 27.2	32.5 31.9	34.0 39.8	26.5 40.3	21.9	132.6	17.7	33.1	34.8
Average of 26 self-incompatible plants															
Average of 22 self-compatible plants															
											0.1	1.7	0.0	3.9	0.4
											20.2	115.8	18.2	34.4	28.4

\*The genotype symbols are explained in the text.

†A = 10 flowers per head following emasculation; B = manipulating entire heads; C = untreated heads (bagged in field).

field results confirmed those obtained in the greenhouse, and they suggest that presence or absence of segregations can be determined easily by field methods.

In general, the different methods used in this study when testing for the self-compatibility allele gave similar results. In every case the difference in average seed set between the self-compatible and self-incompatible plants was highly significant (Tables 1 and 2). Such a difference in averages might be expected, however, due to the large difference between the two original parents. A more important consideration, especially from the standpoint of practical breeding, is which of these tests allowed the most accurate classification of individual plants. Apparently the most critical of the methods was the cross-pollination with parent and  $F_1$  plants. In this case, the tested plants either showed a distinct group reaction or else were equally cross-compatible with all groups.

Almost as good with most plants was the method of selfing 10 flowers per head following emasculation in the greenhouse. All of the self-compatible  $F_1$  plants set as many seeds by selfing as by crossing, whereas the self-incompatibles set practically none. Among the self-compatible  $F_2$  plants, however, a few failed to set seed or set only a low number, so that they could have been classified as self-incompatible if the cross-compatibility test had not been made.

The method of manipulating entire heads was even less satisfactory, since considerable overlapping occurred between the two classes (Table 3). This was in part the effect of manipulation bringing pseudo-self-compatibility into expression with those plants classified as self-incompatible. The least reliable method was that of leaving heads to self-pollinate without any treatment. The large differences between plants in self-pollination without any treatment suggest that autogamy and self-compatibility are distinct characters. The fact that highly significant differences between self-compatible plants were found in average seed set following selfing can be only explained at present by assuming factors which have a strong modifying influence over the  $S_i$  factor under certain conditions. The practical value of the  $S_i$  gene in breeding may depend on the ease with which some of these modifying factors can be eliminated. Even at present, however, the segregations are clear enough for most purposes, and they are found with large-scale field methods.

The results of bagging in the field were complicated not only by the effect of the bagging and manipulation technics, but also by the effect of other environmental influences such as weather. It was not

TABLE 3.—Range in average seed set resulting from selfing individual  $F_2$  plants.

Type of plant	Greenhouse			Field	
	A*	B*	C*	B*	C*
Self-incompatible.	0.0-0.5	0.0-15.0	all 0.0	0.0-13.5	0.0-3.1
Self-compatible...	0.0-38.3	4.6-199.6	0.0-94.3	3.4-79.8	1.9-102.2

\*See Table 2 for explanation of symbols.

possible, of course, to bag all plants on the same day, and consequently some bags were manipulated during fair weather, while rain interrupted the manipulation sequence of others. Superimposed on these sources of variance was the pseudo-self-compatibility of some plants. Finally, one other confusing influence in both the manipulated and unmanipulated series was the male sterility of some plants. This was observed most strikingly among inbred plants.

In the summer of 1940, there were selfed in the field 110 first-generation inbreds which had come from the original self-compatible male parent. Their frequency distribution approximated a normal curve, but it was slightly skewed towards the upper limit (Table 4). Only a few plants equalled their parent in seed set, with the average of all plants (58.6) being considerably lower. Since only four heads per plant were bagged for this experiment, the averages for separate plants may not be extremely accurate, but it is quite evident that modifying factors were segregating to cause this wide spread. It was thought at first that some of the self-incompatible plants at the lower end of the range might be  $S_8S_8$ , having resulted from some  $S_8$  pollen growing far enough to effect fertilization of an  $S_8$  egg.

TABLE 4.—Frequency distribution of 110 first-generation inbreds.

Av. No. seeds per head	0 to 10	10 to 20	20 to 30	30 to 40	40 to 50	50 to 60	60 to 70	70 to 80	80 to 90
Number of plants	6	7	11	7	10	17	14	11	12
Av. No. seeds per head	90 to 100	100 to 110	110 to 120	120 to 130	130 to 140	140 to 150	150 to 160	160 to 170	
Number of plants	3	5	3	2	1	—	—	1	

Mean = 58.6

To test this hypothesis, four plants were selected from the extreme lower end of the distribution. Unfortunately, they flowered very sparsely in the greenhouse, but of the intercrosses among them that were tried most seemed to be compatible, indicating either that different genotypes were involved or that they included  $S_f$ . Three of the plants were then crossed as males with groups 9-III ( $S_8S_8$ ) and 9-IV ( $S_4S_8$ ), and some seed was obtained in every case. None should have been obtained, of course, if the plants were  $S_8S_8$ . Finally, the plants were selfed following emasculation, and in every case either some seed was obtained or the pedicel reflection gave evidence of compatibility.

It seems clear that these plants were not  $S_8S_8$ , but instead bore the  $S_f$  factor. The principal difficulty with making these crosses and selfs, and accordingly the main reason for the questionable results in several cases, was the fact that on all of these four plants the anthers were so hard that by the normal methods of removing pollen none

was obtained. Pollen was secured only by crushing anthers individually with tweezers instead of by tripping the flowers onto a toothpick. The few pollen grains that were obtained in this way were usually moist from other crushed cells, and it was extremely difficult to apply this mixture to the stigma without some damage. In other words, these plants, for all practical purposes, were male sterile, even though they bore the  $S_f$  allele.

One other fact concerning the functioning of the  $S_f$  allele was determined from crosses with  $S_3S_3$  and  $S_4S_4$  plants. The question had arisen whether  $S_f$  was dominant to other alleles borne in the same pistil or, in other words, whether the presence of  $S_f$  would permit  $S_x$  pollen to function in an  $S_fS_x$  pistil despite the oppositional influence of  $S_x$ . The test consisted of crossing  $S_3S_3$  as male onto the two 9-V ( $S_3S_f$ ) plants and  $S_4S_4$  onto the 9-VI ( $S_4S_f$ ) plant. In every case seed was obtained. The number of seeds was on the average somewhat lower than the average of the compatible crosses with these same plants, but only a few heads were used so that the significance of this difference is questionable. It is concluded that in these cases  $S_f$  functions as a dominant or partly dominant allele in affecting pollen tube growth.

Evidence as to the converse proposition, namely, whether the presence of  $S_f$  in a mixture of  $S_f$  and  $S_x$  pollen would stimulate the  $S_x$  pollen to function despite the oppositional influence of  $S_x$  in the pistil, was obtained from the  $F_2$  test. If such stimulation had occurred, some incompatible plants would have been obtained from the cross 9-IV ( $S_4S_3$ )  $\times$  9-VI ( $S_4S_f$ ). Since none were found among the 12 tested in both greenhouse and field and the 5 others tested only in the field, it is evident that in this case the  $S_f$  pollen has no effect in stimulating other pollen to function when the allele borne by this pollen is present in the pistil. Corroborating evidence is the fact that no  $S_3S_3$  segregates were found among the four first-generation inbreds which were tested because of their low seed set in the field.

## DISCUSSION

The main objectives of this study were to determine the nature of the self-compatibility found in the original male parent and to find out enough about its genetic behavior to allow an estimate of its ultimate importance in an inbreeding program. The manner in which it is utilized will depend in large part, of course, on the amount of natural crossing obtained with plants bearing the  $S_f$  allele. For example, if plants bearing this gene will tend in most cases to outcross rather than self, then the factor can be used directly in a breeding program. This gene would be introduced by crossing  $S_f$  plants (preferably homozygous) with selected individuals and the progeny would be selfed as long as desirable, after which vigor could be restored by allowing selected inbreds to outcross under controlled or natural conditions. On the other hand, if  $S_f$  plants tend to self instead of outcross, this factor could be used only indirectly to achieve an end. Such a plan has been suggested for several species. It entails selecting throughout the inbred generations only those individuals heterozygous for  $S_f$ . At any point then, self-incompatibility and vigor

could be recovered in the  $F_1$  simply by crossing two such unrelated inbreds. Further matings on a large scale would then be possible with these self-incompatible  $F_1$  plants.

The former objective has been accomplished in its main outlines. It seems clear that self-compatibility is conditioned here by a single factor,  $S_t$ , which is a member of the multiple allelic series causing self- and cross-incompatibility. Pollen bearing this factor is not inhibited on any styles, including those bearing  $S_t$ , and in certain crosses this factor is dominant to the second allele in plants heterozygous for  $S_t$ . The latter objective has been accomplished, however, only in part. Preliminary evidence suggests that although pollen bearing  $S_t$  will penetrate a style bearing the same allele, it does not compete equally with other pollen bearing different alleles than those present in the pistil. This was seen in the disproportionately low numbers of self-compatible plants segregating out in both  $F_1$  and  $F_2$ . Whether this will obtain also with bee pollination under field conditions is now being tested. It was first pointed out by East (4) that different alleles in the series in *Nicotiana* showed a graded series from complete opposition to almost none. If the same holds for white clover, it may be necessary to determine for each possible self-compatible allele the degree to which pollen carrying it can compete with other pollen in order to judge the usefulness of these alleles.

#### SUMMARY

The original male parent was selected because it was the only plant out of 615 that averaged over 100 seeds per head when self-pollinated under bag in the field in 1938. Except for descendants from this plant, few others have since shown a similar self-compatibility, whereas this high seed set has been duplicated on clones from the original plant by (a) different technics of selfing in the greenhouse, (b) bagging in the field both with and without manipulation, and (c) bee pollination under a cage in the field. In contrast, the female parent showed low pseudo-self-compatibility.

When 14 selected  $F_1$  plants were diallely intercrossed and backcrossed to both parents in the greenhouse, two intra-sterile, interfertile groups of five and six plants, respectively, were found, and these 11 plants were reciprocally compatible with both parents. Different degrees of pseudo-self-compatibility were found among these 11 plants. The other three  $F_1$  plants were cross-compatible with both of these groups, with both parents, and with each other. These three plants were also self-compatible like their male parent under all conditions tested.

These results are explained by postulating that the male parent was heterozygous for a self-compatibility factor ( $S_t$ ), which is a member of the multiple-allelic series conditioning self- and cross-incompatibility, and that this factor was transmitted to the self-compatible  $F_1$  plants.

This theory was confirmed and the different self-compatible genotypes in the  $F_1$  were identified by backcrossing to the parental and  $F_1$  groups, 48  $F_2$  plants resulting from four  $F_1$  intercrosses.



The segregations into self-compatible and self-incompatible individuals were clear in most cases, although the effect of modifying factors was more evident with some methods of testing than with others.

Certain self-compatible individuals were found to be self-pollinating, i.e., autogamous.

Different degrees of self-compatibility were found among 110 first-generation inbreds from the original male parent, but representative plants even from the lower end of the range were shown to bear  $S_f$ . Some of these plants were effectively male sterile because of hard anthers.

By appropriate tests with plants homozygous for the incompatibility alleles, it was shown that  $S_f$  was dominant over the second allele carried with it in a heterozygous plant. Its presence in pollen, however, did not stimulate other pollen applied with it when the latter was inhibited by the same factor in the pistil.

It is pointed out how self-compatibility may be utilized in a breeding program.

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## THE EFFECT OF BORON ON SEED PRODUCTION OF ALFALFA<sup>1</sup>

A. L. GRIZZARD AND E. M. MATTHEWS<sup>2</sup>

THE inconsistency of seed production of alfalfa has long been recognized, but the basic factors responsible for this irregularity have remained somewhat of a mystery. The literature on the subject contains a wide diversity of opinions as to the factors which influence or control the seed set of this plant. Among the factors affecting the seed set of alfalfa which have received the most attention in the past are selection and breeding for increased ability to set seed; tripping of the flowers and factors affecting tripping; soil texture; climatic factors, such as the amount and distribution of rainfall, humidity, and temperature; and fertilizer treatments. During recent years, work has been reported which shows that boron, under some soil conditions, enhances the seed set of alfalfa (1, 2, 4).<sup>3</sup>

Growers of alfalfa in the Southeast have observed for many years that stands of alfalfa persist for only short periods and that yields have been comparatively low, usually being reduced to such an extent that they become uneconomical by the end of the second year. Willis and Piland (5) of North Carolina were the first to call attention to the fact that "alfalfa yellows" in the Southeast could be corrected by the application of small quantities of boron to the soil. This discovery prompted the use of boron as a possible remedy for "alfalfa yellows" at the James City County Station at Williamsburg, Va., in 1939, where Hutcheson and Cocke (3) found that an annual application of 10 pounds of borax per acre gave a 49% increase in the yield of alfalfa. In addition to the increased yield, it was also observed in the summer of 1940 that alfalfa on the plots which received borax blossomed very profusely, while that on the plots which received no boron blossomed very sparingly. This observation indicated that the application of boron to alfalfa soils might increase the seed set of the crop. Work was thus begun to test this point, and the first year's results of this study are herein reported.

### MATERIALS AND METHODS

The results presented in this paper were obtained from a rate of liming and fertilizer experiment. The alfalfa crop was seeded in August, 1939, on Cecil sandy loam soil at Chatham, Va. The plots were 1/40 acre in area and in duplicate. Ground dolomitic limestone was applied to all plots in series 1 at the rate of 2 tons per acre and to all plots in series 2 at the rate of 1 ton per acre before seeding. A 0-20-20 fertilizer was applied at the rate of 1,000 pounds per acre to plots 1 to 3, inclusive, in each series. Plots 4 to 6, inclusive, in each series received 500 pounds per acre of the same fertilizer. Immediately after the first cutting of hay

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 368.

on June 1, 1941, borax at the rate of 15 pounds to the acre was applied to one-half of each plot. When the second cutting for hay was made on July 3, a strip 10 feet wide across each plot was left to determine the effect of the borax treatment on the seed set of the alfalfa.

Two areas 4 by 5 feet were harvested by hand from each plot on August 22. These samples were tied in cheesecloth and air-dried for 2 weeks in an open shed, after which the seed was threshed by rolling the seed pods on a wire screen. The seed was cleaned in a laboratory clover seed cleaner and tested in rag dolls over a period of 24 days for germination.

## RESULTS

The second crop of alfalfa began to bloom about July 1; and by the end of the month, blossoming appeared to be about completed. Therefore, weather records are reported for this period only. During this 31-day period, rainfall was recorded on 16 days, totalling 7.14 inches, with 6.41 inches of this total falling on 15 days during the period from July 1 to 20. The average maximum daily temperature was 87° F. The daily average mean relative humidity at 7:30 a.m. was 82; at 1:30 p.m., 58; and at 7:30 p.m., 74. The percentage of sunshine was 66.

The results presented in Table 1 show that an application of 15 pounds of borax per acre increased markedly the yield of alfalfa hay; average of the two cuttings, July 3 and August 7. This indicates that the soil was deficient in available boron. Increasing the rate of fertilizer applied from 500 to 1,000 pounds per acre increased significantly the yield of alfalfa hay.

TABLE 1.—*Effect of boron on the seed set and yield of alfalfa.*

Fertility treatments	Yield of alfalfa hay, lbs.			Yield of alfalfa seed, lbs.		Germination, %	
	With boron	Without boron	Increase from boron	With boron	Without boron	Soft	Hard
2 tons limestone							
500 lbs. 0-20-20*	3,291	2,573	289	109.7	0.00	71	28
1,000 lbs. 0-20-20*	3,774	3,031	668	132.2	0.00	75	24
1 ton limestone							
500 lbs. 0-20-20†	3,013	2,724	718	131.7	0.00	71	27
1,000 lbs. 0-20-20†	4,360	3,692	743	131.7	0.00	73	26

\*Average of six replicates.

†Average of three replicates.

Alfalfa which received 15 pounds of borax to the acre on June 1, 1941, set a good crop of seed, while that which received no borax failed to set any seed. The largest yield of seed obtained from the borax-treated plots was 184 pounds per acre and the smallest 82 pounds. On land that had received 2 tons of limestone per acre, boron-treated alfalfa which received 1,000 pounds of a 0-20-20 fertilizer per acre gave an increase of 22.5 pounds per acre in yield of seed

over that receiving 500 pounds of the same fertilizer per acre. However, when only 1 ton of limestone was used, the yield of seed for both fertilizer treatments was the same.

These results are in accord with those of other investigators (1, 2, 4). D'iakova (2) in 1938 reported that fertilizers containing boron speeded up the growth and development of alfalfa and also increased the yield of seed. Investigators in Nova Scotia (1) have also found that alfalfa growing in parts of fields where boron had been previously applied to turnips developed more seed and seed pods than that in the rest of the field. Piland and Ireland (4) found that alfalfa which received 20 pounds of borax per acre in the winter of 1941 set a heavy crop of seed, while that which received no borax set no seed. These investigators, however, did not report data giving the actual yield of seed.

Seed from the plants receiving borax gave a germination count (an average of all plots) of 73%; hard seed, 26%; and non-germinable seed, 1%. This is in accord with the results reported by Piland and Ireland (4).

#### DISCUSSION

The results of this investigation suggest the possibility of producing alfalfa seed by the application of borax to certain soils of the eastern part of the United States. It has been frequently observed that under certain favorable conditions, good seed sets were obtained in this area, but that these sets were not consistent. It has therefore been generally assumed that, except in rare cases, weather conditions in the East were unfavorable for the production of alfalfa seed. The data from this experiment show that even in a season of relatively high rainfall, a condition usually thought unfavorable for alfalfa seed production, a fair crop of seed was set when borax was applied to the soil.

Alfalfa commonly makes four cuttings of hay in Virginia. The first two cuttings usually come in May and June during a period in which the occurrence of rainfall is frequent and the hay crops are relatively large. The last two cuttings come in a period in which evaporation is high and the precipitation is light. These conditions do not favor production of large crops of hay, but more nearly typify the conditions under which alfalfa seed is produced in the West. It is thought that with the proper applications of boron to the soil, it might be possible to harvest two cuttings of hay each year and at least one seed crop. This seed crop might be as profitable as the usually two light cuttings of hay and at the same time reduce the drain on the plant nutrients in the soil. Even if only small crops of seed can be consistently produced in the East, it may be possible to improve the plants by selection and hybridization which are best adapted to the soil and climatic conditions of this area.

#### SUMMARY AND CONCLUSIONS

An experiment was conducted on Cecil sandy loam soil at Chatham, Va., in 1941 to determine the effect of boron on the yield and seed set of alfalfa. In these tests, borax applied at the rate of 15 pounds

per acre resulted in an increase in yield of alfalfa from 289 to 743 pounds per acre. The application of borax resulted in the production of 82 to 184 pounds of seed per acre; whereas, the plants on the plots receiving no borax failed to set any seed. This clearly indicates that under the conditions of this experiment, the application of boron in the form of borax had a marked influence on the seed set of alfalfa.

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## DURATION OF RECEPTIVENESS IN CORN SILKS<sup>1</sup>

DONALD F. PETERSON<sup>2</sup>

IT has long been known that corn silks remain receptive to pollen for one and two weeks or even longer after they first appear. A study was undertaken to determine the optimum time of pollination and the rate of decline in receptiveness of the silks. This study was made at the Idaho Branch Experiment Station at Caldwell, Idaho, which is located in the irrigated section of the state. The environmental conditions there are very constant throughout the pollination period. Uniform weather conditions from day to day are desirable for a study of this kind.

### MATERIALS AND METHODS

Silk emergence was recorded on the plants of one vigorous experimental single cross. The hybrid was used in preference to an open-pollinated variety or double-cross to insure the most uniformity of plants. All ear shoots were covered with cellophane bags and after silking, 2 or 3 days, heavy paper bags were placed over the cellophane bags. These were kept intact until the ears were pollinated and left in place until harvest time.

Pollinations were made with large supplies of freshly produced pollen collected from many plants selected indiscriminately throughout the field. Four ears were pollinated each day for a period of 19 consecutive days.

At the time of pollination the silks were cut back to within 1 or 2 inches of the tips of the husks on two of the four ears pollinated each day. This method of silk cutting is a common practice in artificial corn pollination work, especially when the silks have lengthened out considerably. A comparison was made of the receptiveness of silks when cut and not cut by determining the percentage of seed set. Of the uncut group the entire silk length was pollinated.

### EXPERIMENTAL RESULTS

In studying seed setting as affected by cutting and not cutting the silks just prior to pollination, it was found that the results were essentially identical. Because of this similarity, all four ears pollinated each day were handled together with the results summarized as one set of pollinations only.

The mean percentages of seed set resulting from pollination made on 1-day to 19-day-old silks are given in Table 1. Each entry in the table is a mean of three to five ears.

The seed set on 1-day-old silks was 58%. Silks from 2 to 8 days old set seed to the extent of 91% and more with the highest percentage (95) at 5 days. Nine-day-old to 12-day-old silks set about 75% of seed. Silks 14 days old and older resulted in a marked decrease in percent-

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<sup>2</sup>Instructor in Agronomy. The writer wishes to express his appreciation to Dr. H. K. Schultz for his assistance during the course of this study.

TABLE 1.—Mean percentages and coefficients of variability of kernels set on ears when pollinations were made on silks 1 to 19 days old.

Age of silk, days	Seed set, %	Coefficient of variability	Age of silk, days	Seed set, %	Coefficient of variability
1	58.1 ± 5.06	23.1	11	84.0 ± 4.55	10.8
2	91.0 ± 3.60	7.9	12	72.2 ± 14.10	39.1
3	89.5 ± 4.12	11.3	13	*	
4	88.8 ± 5.60	10.9	14	43.0 ± 18.42	74.2
5	95.0 ± 4.35	9.2	15	16.0 ± 4.85	60.6
6	91.3 ± 6.00	11.4	16	5.3 ± 1.50	49.0
7	91.2 ± 6.20	13.6	17	8.2 ± 2.95	71.9
8	93.0 ± 2.83	4.3	18	15.3 ± 4.10	46.4
9	73.8 ± 12.70	34.4	19	7.7 ± 0.50	6.5
10	72.2 ± 12.35	34.2			

\*Due to oversight no pollinations were made.

age of seed set. Nineteen-day-old silks still possessed receptiveness as expressed by a seed set of about 8%.

The standard deviation was calculated for the four ears pollinated each day rather than presenting the percentage of seed set on each ear. These deviations are also presented in percentage of the mean for comparison. Of these values those of the 2- to 8-day period of silk age are relatively small when compared with the other silk age groups. The coefficients of variability are larger in the 9- to 12-day group and still larger in the 14- to 19-day group. Also, the seed set obtained from pollinations made on the first day gave a higher coefficient of variability than the immediately following group. From these results (1 to 19 days) it is seen that as the receptiveness of the silks as expressed by percentage of seed set diminishes, the greater is the variability among the ears pollinated each day.

It is of interest to note the location of seed setting with respect to the ear when pollinations were made on new silks as compared to older silks. This is clearly shown by Fig. 1. It may be seen that the tips of the ears were barren when 1-day-old silks were pollinated and that only the tips carried kernels when very old silks were pollinated.

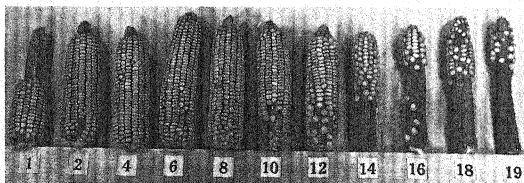


FIG. 1.—Representative ears illustrating differences in receptiveness of silks as expressed by percentage of seed set. The numbers indicate days duration between silk emergence and pollination.

Such a distribution of kernels on the ears<sup>3</sup> is expected if the silks of the butt region of the ear emerge first and those of the tip region last.

#### SUMMARY

The duration of receptiveness in corn silks, as studied by seed setting, extended over a period of more than 19 days. One-day-old silks set seed to the extent of 58%, while 2- to 8-day-old silks set seed rather uniformly, averaging 91%. Seed setting declined thereafter, reaching 8% on 19-day-old silks.

<sup>3</sup>ROBINS, W. W. *The Botany of Crop Plants*. Philadelphia: P. Blakiston's Son and Co. Ed. 3. 1931. (Page 168.)



## POTASH DEFICIENCY SYMPTOMS IN NAPIERGRASS, *PENNISETUM PURPUREUM*<sup>1</sup>

GLENN W. BURTON AND C. L. LEFEBVRE<sup>2</sup>

NAPIERGRASS, *Pennisetum purpureum* Schumach., was introduced into the United States from tropical Africa in 1913 and was received with much enthusiasm by a number of livestock men in Florida and other southern states. This interest gradually lessened due to the poor production obtained in some seasons and the difficulty encountered in maintaining good stands from year to year. In recent years, the failure of Napiergrass in Florida has been reported (1)<sup>3</sup> as due to the eyespot disease caused by *Helminthosporium sacchari* (B. deH.) Butler (2), (*H. ocellum* Faris) and eyespot resistant strains of the grass have been selected. The leaf symptoms caused by *H. sacchari* on Napiergrass have been described in detail by Voorhees (3).

More recently, eyespot-resistant strains of Napiergrass have been developed at the Georgia Coastal Plain Experiment Station, Tifton, Ga., and, when cut to simulate deferred grazing, have outyielded the old eyespot susceptible strains from 3 to 7 times. These strains also have survived adverse winters at Tifton, Ga., much better than the disease-susceptible strains. However, irregular brown spots have developed on the leaves of some of the strains known to be resistant to eyespot disease and in some cases firing of the leaf tips and margins have been observed. The studies reported in this paper show that these symptoms are due to potash deficiency and can be prevented by the application of muriate of potash to the soil.

### EXPERIMENTAL

Small brown spots on the leaves of some of the strains of Napiergrass known to be resistant to eyespot were noted in 1938. These spots were approximately the same size and color as the eyespot lesions but were irregular in outline (Figs. 1 and 2). The irregular or indefinite outline of these lesions proved upon close examination to be distinctly different from the usually elliptical sharply defined borders of the lesions caused by the eyespot fungus. Frequently, the tips and margins of these spotted leaves were "fired" as indicated in Fig. 1. Numerous attempts were made to isolate organisms from the discolored leaves during various stages of development, but it was impossible to obtain fungi from the younger, small lesions that were surrounded by green, healthy tissue. From old lesions, however, where the leaves were "fired" and dried up, several species of *Helminthosporium* and *Curvularia* were obtained. However, *H. sacchari*, the cause of eyespot, was not isolated from these lesions at any time. When these isolates were used in inoculation trials, they were found to be nonpathogenic, indicating that the disease was of a non-parasitic nature, and that the fungi were secondary invaders.

<sup>1</sup>Cooperative investigations at Tifton, Ga., of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, the Georgia Coastal Plain Experiment Station, and the Georgia Agricultural Experiment Station. Received for publication December 6, 1941.

<sup>2</sup>Geneticist and Associate Pathologist, respectively, U. S. Dept. of Agriculture.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 375.

In 1939 velvetbeans following a 2-year old Napiergrass breeding block developed very severe potash deficiency symptoms which were corrected with the application of muriate of potash at the rate of 150

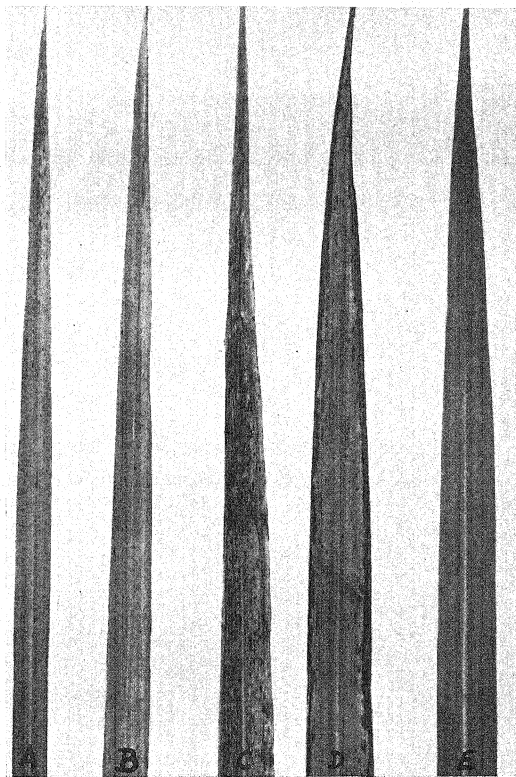


FIG. 1.—A and B, Napiergrass leaves infected with *Helminthosporium sacchari*; C and D, potash deficiency symptoms in Napiergrass leaves; E, normal Napiergrass leaf.

pounds per acre. In the same year, rows of velvetbeans adjacent to a planting of Napiergrass spotted badly indicating that the Napiergrass had exhausted the available potash in the soil within 8 to 10 feet of it. This spotting was likewise corrected with the application of potash. These results suggested that the symptoms observed on Napiergrass might be due to potash deficiency so the following test was made.

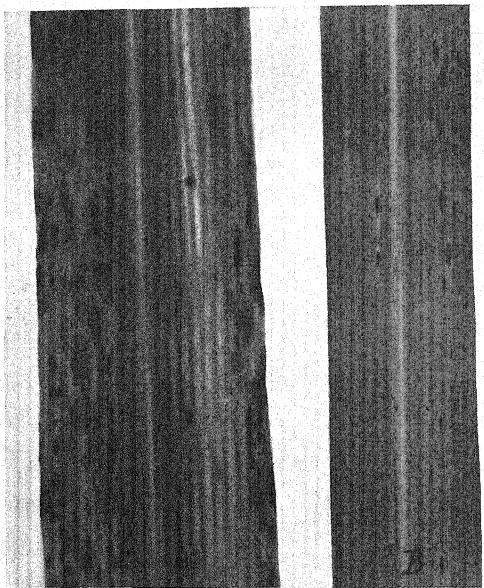


FIG. 2.—A, Napiergrass leaf showing typical potash deficiency symptoms; B, Napiergrass leaf showing the eyespot caused by *Helminthosporium sacchari*.

A replicated rod row test of a number of Napiergrass selections was planted in 1941 on land known to be rather low in available potash. Some selections, particularly the very leafy ones, developed leafspot and leaf-firing symptoms much earlier and to a greater extent than others. The application of muriate of potash at a rate of 200 pounds per acre stimulated the formation of normal leaves for

the remainder of the season, thus showing that the spotting and firing of the leaves was due to potash deficiency. Several of these strains have been grown for the past two years on land well supplied with potash and have not developed the symptoms described here as characteristic of potash deficiency.

The observations made at Tifton, Ga., indicate that no relation exists between potash deficiency and the occurrence of *H. sacchari* in Napiergrass. Disease-resistant strains of Napiergrass are no more susceptible to *H. sacchari* on potash-deficient soils than on soils well supplied with potassium. The occurrence of heavy epidemics of *H. sacchari* in well-fertilized plantings of disease susceptible Napiergrass suggests that soil fertility will not materially alter the relation between the host and the pathogen.

#### SUMMARY

1. In Napiergrass the formation of irregular brown spots on the leaves and sometimes "firing" of the leaf tips and margins supplies a reasonably dependable index of potassium starvation.

2. Napiergrass, probably because of its tremendous leaf production, has a high potash requirement and must be supplied with rather large quantities of potassium in order to avoid the loss of leaves due to potash deficiency.

3. That Napiergrass is an unusually strong feeder for potassium is indicated by the severe potash-deficiency symptoms developed by velvetbeans following it in rotation.

4. Very leafy strains of Napiergrass developed potash-deficiency symptoms earlier and to a greater degree than the less leafy types.

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EFFECTS OF SELF-POLLINATION IN SWEET CLOVER<sup>1</sup>E. E. HARTWIG<sup>2</sup>

SWEET clover is one of the most widely grown legumes in the corn belt states for soil improvement and pasture purposes. The elimination of certain undesirable features, however, would enhance its value for forage purposes. The need for later maturing, more palatable, disease-resistant strains has stimulated interest in the improvement of the crop. However, a better understanding of the effect of selfing on *Melilotus* is essential in determining the methods of procedure in a breeding program.

Previous studies on the pollination habits of *Melilotus alba* have been somewhat controversial; some workers believe it sets seed freely without artificial manipulation, while others believe that crossing is necessary. However, most workers are agreed that in the species *M. officinalis* crossing or some form of manipulation of the flowers is necessary for seed production.

This study was conducted to obtain additional information upon the effect of selfing and methods of pollination in several biennial species of *Melilotus* by a comparison of progenies from open-pollinated and self-pollinated seed of individual plant selections.

## LITERATURE REVIEW

Darwin (4)<sup>3</sup> referred to *M. officinalis* as a plant requiring cross-pollination to set seed. Kirchner (7) concluded that *M. alba* is generally self-pollinated and that cross-pollination is unnecessary. Coe and Martin (2) carried on a number of experiments at Ames, Iowa, and Arlington, Va., with *M. alba* in which they covered the flowers with tarlatan bags. The average seed set of covered racemes was 2.9%, while 66.51% of the flowers of uncovered racemes set seed.

Kirk (8) and Elders (5) carried on experiments in both *M. alba* and *M. officinalis* to determine the amount of seed produced when insect visitations were prevented. Both workers concluded that *M. alba* set seed readily when insects were excluded, but in *M. officinalis* very few seeds were produced under these conditions. Brink (1) considered *M. officinalis* to be highly self-sterile. Dann (3) concluded that *M. alba* is only slightly self-pollinating but highly self-fertile, while *M. officinalis* is non-self-pollinating and also self-sterile. Ufer (14) studied the question of seed setting in several species of *Melilotus*. Of the biennial species, *M. wolgica* gave excellent production of selfed seed. No seed was obtained when *M. dentata* or *M. suaveolens* was selfed. The species *M. alba*, *M. officinalis*, and *M. altissima*

<sup>1</sup>Contribution from the Department of Agronomy, Illinois Agricultural Experiment Station, Urbana, Ill., in cooperation with the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture. Published with the approval of the Director of the Station. Part of a thesis submitted in partial fulfillment of the Ph.D. degree in Agronomy, University of Illinois. Received for publication December 29, 1941.

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 387.

showed considerable interplant variations, but in general *M. alba* set more seed upon self-pollination than did *M. officinalis*. Ufer's work was principally with plants and varieties of *M. alba* and *M. officinalis*.

Kirk and Stevenson (11) investigated the causes of interplant differences with regard to spontaneous self-fertilization in six varieties of sweet clover. Factors considered to be involved were length of stamens, stage of flower development when pollen is liberated from the pollen sacs, distribution of free pollen within the flower, size of cavity in the upper part of the keel, amount of pollen, condition of pollen, and receptivity of stigma. Spontaneous self-fertilization was considered to be a rare occurrence in *M. officinalis*. The authors considered the production of spontaneously self-fertilized strains of *M. alba* to be possible and very desirable from the standpoint of maintaining purity of improved varieties and also of increasing seed yields.

Elders (6) and Kirk (9) grew plants of the Alpha type adjacent to plants of the common type sweet clover. Since the Alpha type is a simple recessive to the common growth habit,  $F_1$  plants could easily be recognized. Kirk found the average percentage of cross-pollination on 13 Alpha plants to be 57%. Over 50% crossing occurred in all but three plants. Elders found 8% crossing in 1925 and 23% in 1927. Smith (12) grew annual and biennial plants in adjacent rows. Of 227 plants grown from seed taken from biennial plants, 126 were annual. He concluded that the chance for intervarietal crossing equaled that for intravarietal crossing.

Kirk (10) does not consider the loss of vigor by inbreeding to be a disturbing factor. In discussing strain building as a means of improving cross-fertilized crops, Stevenson (13) states that in the biennial white blossom sweet clover the loss of vigor upon selfing is relatively small but sufficient to reduce the productivity of the new lines below that of the parent variety.

#### EXPERIMENTAL PROCEDURE AND MATERIALS

Late-flowering, disease-free plant selections were made in 1937 and 1938 from varieties and introduced strains as a part of the sweet clover improvement program. One or more branches of each selected plant were covered with a 9-inch by 18-inch fine-mesh cotton bag to prevent insect visitations. Self-pollination of the bagged flowers was aided by rolling the bag between the palms of the hands twice each week as new flowers appeared. At maturity both open-pollinated and self-pollinated seed were harvested.

Progenies of 1937 selections were grown during the seasons of 1938 and 1939, and the progenies of the 1938 selections plus a few 1937 selections were grown in 1939 and 1940. Further advanced selfed generations were produced in the greenhouse during the winter months by proper control of the temperature and artificial light. These generations were random samples, since no selection for plant characteristics was made in the greenhouse. Flowers were manipulated to aid pollination.

Open-pollinated and self-pollinated progenies from selections were grown in adjacent blocks in the field. The plants were spaced 2 feet apart in rows 40 feet long and 40 inches apart. The number of plants grown from O. P. or  $S_1$  seed ranged from 20 to 80 plants from each mother plant selection. Ten to 20 plants were grown from each  $S_2$  and  $S_3$  line.<sup>4</sup> Seedlings were started in the greenhouse and transplanted to the field, or the seed was planted in rows in the field and the seedlings later thinned to the proper spacing.

<sup>4</sup>O. P. = Open-pollinated;  $S_1$  = First selfed generation;  $S_2$  = Second selfed generation;  $S_3$  = Third selfed generation.

This study included comparisons in 1939 of 13 *M. officinalis* and 49 *M. alba* families; and in 1940 comparisons of 1 *M. taurica*, 2 *M. suaveolens*, and 40 *M. alba* families. Data in this paper are reported on mature height, stem number, date of first bloom, seed production, and disease reaction.

## EXPERIMENTAL RESULTS

## MATURE HEIGHT

All plants were measured after they had made their full second-year growth. The various families within each variety and species differed in the comparative heights of O. P. and  $S_1$  plants. The mean height for each  $S_1$  progeny has been expressed as a percentage of its O. P. sibs. The frequency distribution of these relationships is given in Table 1, along with the means for the  $S_1$  progenies for each variety and species for each year. The difference in height between O. P. and  $S_1$  sibs was statistically significant in each of the varieties and in all species. In two families, one in *M. officinalis* and one in *M. alba*, the mean height of the  $S_1$  progeny was only 51% as great as the mean height of the O. P. sibs. In general, the *M. officinalis* families gave somewhat greater differences than were found between O. P. and  $S_1$  progenies in *M. alba*.

TABLE 1.—Height of the first selfed generation of sweet clover families expressed as a percentage of their open-pollinated sibs.

Variety or species	Year	Number of pro- genies	Mean height, %	Number of families and class centers in %															
				51	63	66	69	72	75	78	81	84	87	90	93	96	99	102	
<i>M. alba</i> (Spanish)	1939	9	85.7				1		1			2		4	1				
	1940	9	86.6						1	1	3	1	1	1		1			
Iowa Late	1939	10	89.1							1	3	1	2	2		1			
	1940	6	79.7		1		1		1				1			1			
Evergreen	1939	10	89.6							1	2	1	3	1		2			
	1940	7	78.3	1			1	1	1			1		1	1		2		
Sangamon	1939	5	82.4			1				1		1				1			
	1940	2	76.0		1									1					
Misc. <i>M. alba</i>	1939	5	88.9							1		3					1		
	1940	4	92.0								1		1		1	1			
Ill. com- mercial	1939	10	78.6				2		2	1	2	3							
All <i>M. alba</i>	1939	49	85.7			1	3		3	2	5	11	6	9	4	1	3		
	1940	28	83.1	1	2		2	1	2	1	2	4	2	4	2	3	2		
<i>M. officinalis</i>	1939	13	72.6	1	1	1	1	2	3	3				1					
<i>M. taurica</i>	1940	1	96.0													1			
<i>M. suaveo- lens</i>	1940	1	92.4													1			

S<sub>2</sub> lines were grown from 17 *M. alba* and 3 *M. officinalis* families in 1939 and from 1 *M. taurica*, 2 *M. suaveolens*, and 26 *M. alba* families in 1940. In every family the mean height for the S<sub>2</sub> progenies was reduced below that of the S<sub>1</sub>. The mean height decrease from S<sub>1</sub> to S<sub>2</sub> progenies was approximately 10%. Twelve S<sub>3</sub> progenies were grown from six S<sub>2</sub> lines in 1940. Each progeny showed a further decrease in height with a mean decrease of 15%. These advanced selfed progenies were random samples from the preceding selfed population.

Table 2 gives a comparison of O. P., S<sub>1</sub>, and S<sub>2</sub> populations grown in 1939, along with comparisons between S<sub>1</sub> and S<sub>2</sub>, and between S<sub>2</sub> and S<sub>3</sub> populations grown in 1940. Fig. 1 illustrates graphically the frequency distribution of O. P., S<sub>1</sub>, and S<sub>2</sub> plants for mature height.

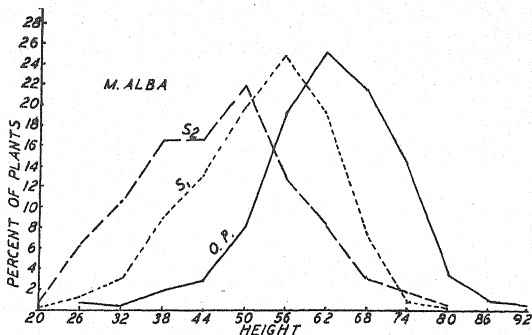


FIG. 1.—Frequency distribution of O. P., S<sub>1</sub>, and S<sub>2</sub> plants of *M. alba* for height, 1939.

TABLE 2.—Height comparisons between O. P. and selfed populations of *M. alba*.

Populations compared	Generation	Number of progenies	Number of plants	Mean height in inches and standard error	%
O. P., S <sub>1</sub> , and S <sub>2</sub> populations of <i>M. alba</i> , 1939	O. P.	17	676	62.7±0.38	100
	S <sub>1</sub>	17	584	52.7±0.41	84
	S <sub>2</sub>	31	386	48.4±0.59	74
S <sub>1</sub> and S <sub>2</sub> populations of <i>M. alba</i> , 1940	S <sub>1</sub>	21	704	42.0±0.38	100
	S <sub>2</sub>	53	747	39.9±0.36	95
S <sub>2</sub> and S <sub>3</sub> populations of <i>M. alba</i> , 1940	S <sub>2</sub>	7	110	43.3±1.02	100
	S <sub>3</sub>	12	159	35.8±0.66	83



## STEM NUMBER

In obtaining a measure of loss of vigor through selfing, it was considered advisable to supplement height data with counts of the number of primary stems. Stems arise from crown buds early in the spring and their number remains constant throughout the season. Stems were counted late in the season after the plants had lost their lower leaves. Number of stems on 20 plants, where available, were counted for each lot of O. P. and  $S_1$  plants. Where more than 20 plants were available, the first 20 consecutive plants were used. Results showed very little reduction in stem number as a result of selfing. *M. officinalis* showed somewhat greater decrease in stem number than did any of the other species observed. Frequency distributions of stem number in open-pollinated and selfed generations of sweet clover are shown in Table 3.

TABLE 3.—Frequency distribution and comparison of stem number in open-pollinated and selfed populations of sweet clover.

Species and year	Generation	Number of plants	Frequency distribution of plants with number of stems indicated								Mean number of stems and standard error
			8	14	20	26	32	38	44	50	
<i>M. alba</i> 1939	O. P.	368	16	95	130	90	22	14	1	—	20.9±0.35
	$S_1$	310	31	67	102	72	26	9	3	—	20.7±0.42
	$S_2$	423	55	97	126	107	31	7	—	—	19.8±0.34
<i>M. officinalis</i> 1939	O. P.	155	6	33	50	41	16	7	—	2	22.6±0.54
	$S_1$	125	18	34	31	24	10	6	1	1	19.6±0.87
<i>M. taurica</i> 1940	$S_1$	17	—	2	4	7	2	2	—	—	25.3±1.64
	$S_2$	30	1	4	5	12	7	1	—	—	24.6±1.25
<i>M. suaveolens</i> 1940	$S_1$	49	7	17	10	11	4	—	—	—	18.5±1.03
	$S_2$	67	8	32	17	4	5	1	—	—	17.2±0.82
<i>M. alba</i> 1940	$S_1$	359	27	69	78	96	53	24	7	5	23.4±0.48
	$S_2$	546	25	105	116	147	86	43	17	7	24.3±0.39
<i>M. alba</i> 1940	$S_2$	77	13	25	13	14	8	4	—	—	19.2±1.03
	$S_3$	122	29	32	35	17	3	3	3	—	17.7±0.76

## DATE OF FIRST BLOOM

Date of first bloom was recorded at regular intervals. A distinct difference was observed between flowering dates of O. P. and  $S_1$  progenies. The mean date of first bloom of each  $S_1$  progeny was from 2 to 6 days later than that of the corresponding O. P. progeny in *M. alba* and *M. officinalis*. The differences in flowering dates between O. P. and selfed generations are illustrated in Figs. 2 and 3. Fig. 2 shows graphically the distribution of O. P. and  $S_1$  plants of *M. officinalis*, while Fig. 3 shows the distribution of O. P.,  $S_1$ , and  $S_2$  plants of *M. alba* with respect to date of first bloom. Of 78  $S_2$  *M. alba* progenies grown, 32 bloomed earlier than the parent  $S_1$ , 6 were the

same, and 40 were later. Of 12  $S_3$  lines, 5 were earlier and 7 later than the parent  $S_2$ . The behavior of these advanced selfed progenies with regard to date of first bloom eliminates the possibility of attributing the difference of flowering date in O. P. and  $S_1$  progenies to loss of vigor.

The mother plants from which these populations were grown were the latest flowering plants in the populations from which they were selected, making up approximately 2% of the total population. Therefore, on the basis of chance, when crossing occurred, the male parent would usually have been earlier than the plant selected. The results obtained in comparing open-pollinated and  $S_1$  progenies for time of maturity indicate that a considerable amount of crossing occurred and that early maturity is either dominant or intermediate in its inheritance. Later data obtained from controlled crosses of early with late-flowering parents gave evidence that early maturity is partially dominant to late maturity.

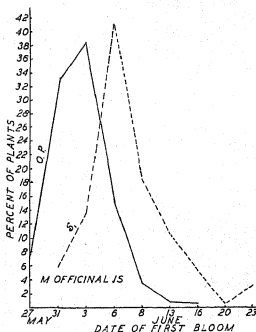


FIG. 2.—Frequency distribution of O. P. and  $S_1$  plants of *M. officinalis* for date of first bloom.

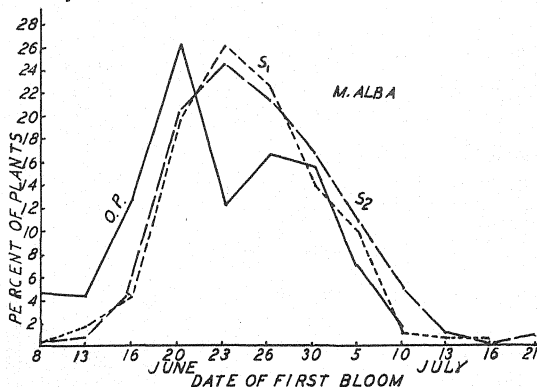


FIG. 3.—Frequency distribution of O. P.,  $S_1$ , and  $S_2$  plants of *M. alba* for date of first bloom.

## SEED PRODUCTION

As the plants approached maturity, notes were taken on seed production. Plants were rated on a numerical basis. Five classes were

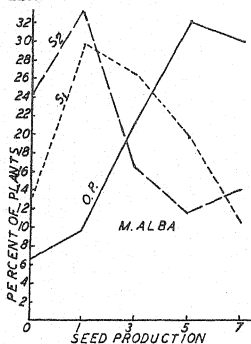


FIG. 4.—Frequency distribution of O. P., S<sub>1</sub>, and S<sub>2</sub> plants of *M. alba* for seed production classes.

were good seed producers, while in *M. suaveolens* they were uniformly poor.

## DISEASE REACTION

In 1939 all plants of *M. alba* were classified for degree of stem blackening shortly before maturity of the plant. The exact cause of this disease was not determined, but it is believed to be a result of the interaction of several fungi inhabiting the outer layers of the sweet clover stem. This condition apparently interferes with normal maturity, and when severe, only immature, non-viable seeds are produced. Plants were classified on a scale of 1 to 9. A rating of 1 indicates that the plant is nearly free from blackening, while a rating of 9 indicates that the stem is almost completely blackened.

The distribution of plants in O. P. and S<sub>1</sub> populations is shown in Table 5. Table 6 shows the distribution of O. P., S<sub>1</sub>, and S<sub>2</sub> plants. A higher percentage of the selfed plants was given a rating of 7 or over than of the plants from O. P. seed, indicating a higher degree of stem infection in the selfed plants.

In 1940, black stem infection was not great enough to make classification possible. It was observed that uniformity was being approached for disease reaction other than for stem blackening in S<sub>2</sub> lines. For example, of four S<sub>2</sub> lines grown from one S<sub>1</sub>, one was completely susceptible to infection by *Ascochyta caulicola*, two showed segregation, and the fourth line was free from disease. Other cases were observed in which all plants of one or more S<sub>2</sub> were dead from

used, namely, 0, no seed; 1, poor; 3, fair; 5, good; 7, extra good. While it is realized that this method is not exact, it facilitates classifying a large number of plants for a variable character.

The frequency distribution of O. P. and S<sub>1</sub> plants for production of seed is reported in Table 4. Fig. 4 illustrates comparisons graphically among O. P., S<sub>1</sub>, and S<sub>2</sub> populations for seed production.

The fact that a higher percentage of plants from selfed seed was rated as poor seed producers indicates that they lack the capacity to produce seed, even though they flowered profusely and pollinating insects were present.

Little difference was apparent between plants of the different generations in *M. taurica* and *M. suaveolens*. In *M. taurica* all plants

TABLE 4.—Frequency distribution of plants for seed production in open-pollinated and first generation selfed sweet clover.

Variety and species	Generation	Total No. of plants	No. of plants in class*					% plants scoring	
			0	1	3	5	7	3 and under	5 and over
<i>M. officinalis</i>	O. P.	378	22	17	33	182	124	19.0	81.0
	S <sub>1</sub>	291	84	127	45	33	2	88.0	12.0
<i>M. alba</i> Spanish	O. P.	392	19	44	80	117	132	36.5	63.5
	S <sub>1</sub>	370	37	111	89	88	45	64.0	36.0
Iowa Late	O. P.	399	41	57	118	108	75	54.1	45.9
	S <sub>1</sub>	368	82	101	94	58	33	75.3	24.7
Illinois Commercial	O. P.	307	28	57	92	79	51	57.7	42.3
	S <sub>1</sub>	326	115	100	78	20	13	89.9	10.1
Evergreen	O. P.	351	8	37	91	118	97	38.8	61.2
	S <sub>1</sub>	157	21	26	52	40	18	63.1	36.9
Sangamon	O. P.	219	10	14	53	68	74	35.2	64.8
	S <sub>1</sub>	101	9	11	35	23	20	54.5	45.5
All <i>M. alba</i>	O. P.	1,970	145	256	511	551	507	46.3	53.7
	S <sub>1</sub>	1,553	288	434	395	259	177	71.9	28.1

\*0 = No seed; 1 = Poor; 3 = Fair; 5 = Good; 7 = Extra good.

TABLE 5.—Frequency distribution of plants for degree of stem blackening in open-pollinated and first generation selfed *M. alba*.

Variety	Generation	Total No. of plants	No. of plants in class*					% plants scoring	
			1	3	5	7	9	5 and under	7 and over
Spanish	O. P.	379	1	40	120	202	16	42.5	57.6
	S <sub>1</sub>	340	—	19	75	219	27	27.6	72.4
Iowa Late	O. P.	378	10	55	93	135	85	41.8	58.2
	S <sub>1</sub>	350	—	51	92	93	114	40.8	59.2
Ill. Commercial	O. P.	300	24	65	130	69	12	73.0	27.0
	S <sub>1</sub>	288	16	45	106	96	25	58.0	42.0
Evergreen	O. P.	353	3	132	135	80	3	76.5	23.5
	S <sub>1</sub>	154	—	48	66	37	3	74.0	26.0
Sangamon	O. P.	209	3	89	99	18	—	91.4	8.6
	S <sub>1</sub>	98	2	63	23	10	—	89.8	10.2
All <i>M. alba</i>	O. P.	1,853	47	478	631	538	159	62.4	37.6
	S <sub>1</sub>	1,419	18	295	424	505	177	51.9	48.1

\*A rating of 1 indicates almost complete freedom from blackening, while a rating of 9 indicates stem almost completely blackened.

undetermined causes prior to the time of normal maturity, while sister  $S_2$  selections appeared to be free from disease.

### DISCUSSION OF RESULTS

Investigations reported in this paper dealing with the effects of self-pollination in *M. officinalis* have shown a decided decrease in vigor when  $S_1$  plants are compared with their open-pollinated sibs. Loss of vigor was expressed by a decrease in height, stem number, and ability to produce seed even when pollinating insects were plentiful. The decreased vigor of  $S_1$  plants along with the difference in flowering dates of open-pollinated and  $S_1$  sibs clearly indicates that in *M. officinalis* cross-pollination is the general rule.

Selfing *M. alba* has given results very similar to those of selfing *M. officinalis*. By analogy we may then conclude that *M. alba* is also highly cross-pollinated. Since loss of vigor accompanied selfing in all comparisons made, it seems highly improbable that a high-yielding spontaneous self-fertilizing strain as described by Kirk and Stevenson (11) may be isolated from any of the material used in this investigation.

The results obtained in this study are more likely to underestimate than to overestimate the extent of natural crossing. Under field conditions, each plant produces only 3 or 4 stems as compared with 8 to 50 stems for spaced nursery plants. These large spaced plants provide a much greater opportunity for natural selfing than is likely under solid seeding conditions. It is also highly probable that under solid seeding conditions less vigorous plants growing from selfed seed will be crowded out as a result of competition.

Many  $S_2$  lines of *M. alba* were found to be quite uniform for time of first bloom and disease reaction. Furthermore, marked differences were noted between  $S_2$  lines tracing to the same mother plant selection. Therefore, in an improvement program, selection among and within  $S_2$  lines should give very satisfactory results. The best inbred lines thus produced can be combined into a synthetic variety. Since natural crossing seems to be the general rule, a distribution of favorable growth factors will likely be maintained in the synthetic variety.

A procedure which should give rapid progress in the improvement of common biennial white blossom sweet clover and also utilize the findings of this investigation may be outlined as follows:

Selfed seed should be obtained from mother plants having as many desirable characters as possible in material of diverse origin.  $S_1$  seed obtained from these plants may be planted in the greenhouse in the fall, and by properly controlling the temperature and artificial light,  $S_2$  seed will be produced in sufficient time for spring planting in the field. This procedure allows the  $S_2$  populations to be grown 2 years earlier than if the  $S_1$  plants were grown in the field. In order to reach a compromise between adequately testing the progeny of a few mother plants and testing many mother plants, it is suggested that 10  $S_1$  plants be grown in the greenhouse from each mother plant selected. Since many of the  $S_2$  lines will be quite uniform, 10 or 12 plants should be sufficient to test the line in the field nursery for its growth habit,

time of flowering, and disease reaction. From 20 mother plant selections, 200  $S_1$  plants will be grown in the greenhouse, which will give 200 different  $S_2$  lines and 2,000 to 2,400 spaced  $S_2$  plants in the field nursery.

The most desirable  $S_2$  plants from the best lines should be selfed, or where the line is uniform, it may be sibbed by caging and enclosing honey bees. If the seed supply is small, particularly following selfing, it may be increased by sib-pollination with honey bees in the greenhouse. The various inbred lines should then be grown in the field, both as spaced plants and in solid rows, and all inferior lines discarded. The remaining lines may then be tested in diallel crosses; these crosses may be made either by hand or with honey bees in the greenhouse. It is essential that an  $F_2$  population be grown from each cross; lines giving crosses which segregate for any of the major characters for which selection was made should not be used in the synthetic variety. Remnant  $F_2$  seed from the selected crosses can then be grown and the plants intercrossed with the aid of honey bees to initiate the synthetic variety. Concurrently with testing the inbred lines in diallel crosses, a bulk cross may be made, and increased, using all of the inbred lines. Then, should there be no segregation in any of the  $F_2$  populations serious enough to justify discarding the cross, several years' time will be gained in producing the synthetic variety. Should such segregation occur, however, this phase of the work should be discarded.

TABLE 6.—Frequency distribution of O. P.,  $S_1$ , and  $S_2$  plants of *M. alba* for degree of stem blackening.

Generation	Total No. of plants	No. of plants in class					% plants scoring	
		1	3	5	7	9	5 and under	7 and over
O. P.....	682	32	193	304	133	20	77.6	22.4
$S_1$ .....	557	16	100	203	215	23	57.3	42.7
$S_2$ .....	365	8	75	108	144	30	52.4	47.6

At the present time improvement in plant characters is considered more essential in the synthetic variety than increased yield of forage. However, it is desired that the forage yield of the synthetic variety should at least equal that of the present varieties. The difficulty encountered in obtaining selfed seed after two or three generations of selfing precludes the establishment of lines of sweet clover as uniform as corn inbred lines now desired by the corn breeders. However, sweet clover lines produced as described above can be expected to be fairly homozygous for a few characters such as disease reaction, time of maturity, and coumarin content.

Evidence to the effect that early maturity is partially dominant to late maturity is very encouraging from the standpoint of maintaining the purity of late-maturing varieties after they are produced. Seed fields can be effectively rogued of all early-flowering plants before the bulk of the plants begin flowering, thus eliminating crossing with the

early type. All commonly grown sweet clover is sufficiently early to enable recognition of crosses with the late strains when they occur.

### SUMMARY

Open-pollinated and selfed populations of four biennial species of *Melilotus* were grown in adjacent blocks and compared for mature height, stem number, date of first bloom, seed production, and disease reaction

Comparisons in *M. officinalis* showed  $S_1$  plants to be lower in height, ranging from 10 to 49% when compared with their open-pollinated sibs, with a mean decrease of approximately 30%. Similar comparisons with open-pollinated sibs in *M. alba* showed a decrease for the  $S_1$  plants ranging from 1 to 49% with a mean decrease of approximately 16%. A further decrease was apparent in  $S_2$  and  $S_3$  generations.

Stem number of selfed plants of *M. officinalis* was slightly lower than for open-pollinated plants. No consistent or significant decrease in stem number was shown following selfing in *M. alba*.

The mean date of first bloom of  $S_1$  progenies was 2 to 6 days later than the mean date of first bloom of open-pollinated sibs when the mother plants were selected for late flowering. In *M. alba* the mean date of first bloom of  $S_2$  progenies approximated the date of first bloom of the parent  $S_1$ , and similarly close agreement was shown between  $S_3$  progenies and parent  $S_2$  plants. Consequently, the difference in date of first bloom between open-pollinated and  $S_1$  material cannot be explained as a result of loss of vigor, but rather as a result of crossing between the selected plant and earlier plants. Some  $S_2$  lines showed considerable uniformity for time of first bloom.

Plants grown from open-pollinated seed were superior to  $S_1$  plants with respect to seed production. In *M. officinalis* 88% of the  $S_1$  plants were classified as poor seed producers, while 81% of the open-pollinated plants were classified as good seed producers. In *M. alba* approximately 60% of the open-pollinated plants were classed as good seed producers, while only 30% of the  $S_1$  plants were similarly classified. A higher percentage of good seed-producing plants was found in the  $S_1$  than in the  $S_2$  generation.

Selfed plants showed a slightly higher degree of black stem infection in 1939 and mortality of selfed plants was somewhat greater in each of the years 1939 and 1940 than for the open-pollinated plants. Also, in 1940, some  $S_2$  lines appeared to be uniform in resistance and others in susceptibility to disease attack.

The differences shown between open-pollinated and selfed progenies of both *M. officinalis* and *M. alba* were great enough to conclude that both species are highly cross-pollinated in nature. Although only relatively few plants were investigated in *M. taurica* and *M. suaveolens*, the differences observed between open-pollinated and selfed plants indicated that in these species, too, crossing is an important factor.

A procedure for developing a synthetic variety is suggested.

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## NOTES

**"FLOSS", A SUGGESTED TERM TO DESIGNATE A MIXTURE  
OF MATERIALS**

IN the repair of injuries to many types of turf it has long been a custom of golf course superintendents, athletic field ground keepers, polo field caretakers, and others in charge of turf areas to use a mellow mixture of soil, sand, humus, lime, fertilizer, and seed. The mixture is pressed down firmly into the injured area and in a relatively short time the grass seedlings appear and tend to heal the scar and to reestablish a uniform appearance. Home owners have been urged to adopt this practice.

One of the difficulties in encouraging wide adoption of this excellent practice is the wordiness of the description of the material. Various descriptions which have been used are "Seed-soil mixture," "Fertilizer-lime, seed-soil mixture", and similar expressions. Golf course superintendents mix "topdressing" with the seed, but it is not used as a "topdressing" so that this term is not applicable, especially since many other turf caretakers do not have a similar material.

In the course of construction of the Pennsylvania Turnpike, the writer was asked to assist with the program of establishing stabilization cover on the banks and other raw exposed areas. The use of new and novel seeding equipment necessitated using a mixture containing fertilizer, lime, organic matter, soil, and seed. The principle involved in the mixture is similar to that of repairing scars in established turf.

It occurred to the writer that the first letter of each of the words spelled the word "FLOSS", as follows:

Fertilizer  
Lime  
Organic matter  
Soil  
Seed

Since the word FLOSS is a common word known to all, it seemed that it would not be amiss to use it in this connection to simplify the description of a widespread practice. It is suggested that the word be written in capital letters, FLOSS, so that the printed word would be distinctive and would not be confused with other conceptions of the word.

Therefore, it is suggested for the sake of clarity and brevity that the word "FLOSS" be used to designate complete mixtures of fertilizer, lime, organic matter, soil and seed, or mixtures of seed with any combination of the other ingredients. It is recognized that some soils are abundantly supplied with calcium and hence may not need added lime. This in no wise invalidates the term "FLOSS", since it is used to designate the complete mixture regardless of whether the lime is natural in the soil or is added during mixing. This holds for other ingredients except seed. If no seed is contained in the mixture, then it is not "FLOSS" but simply a mixture of materials.—FRED V. GRAU, *Pennsylvania State College, State College, Pa.*

SEED PRODUCTION BY KUDZU (*PUERARIA THUNBERGIANA*) IN  
THE SOUTHEASTERN UNITED STATES DURING 1941

EXTENSIVE observations were made of Kudzu seed production in the fall of 1941 during a seed-collecting campaign covering Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, and Mississippi. Scouting for seed was done by workers of the Soil Conservation Service, U. S. Dept. of Agriculture. Harvesting was done mainly by SCS-CCC camps. Approximately 2,400 pounds of hulled seed were collected for seedling production in the Soil Conservation Service nurseries in 1942. Most of the Kudzu seed previously used had been imported from Japan.

Nearly all of the seed collected in 1941 came from vines growing over bushes, trees, fences, or other vertical supports. In a few fields, fair to good crops of seed were produced without vertical supports for the vines.

For the region covered, seed production was better in the northern half than in the southern half. No seed were found in Florida. Seed production was generally good in North Carolina and locally good in the remaining five states. In 1940 seed production was generally poor in North Carolina.

Old Kudzu vines produced more seed than young vines. Very few seed were produced by plants under 3 years of age.

No relation between color of foliage and seed production was evident in North Carolina and Virginia. Further south, a number of observers noted an inverse relation between dark-green foliage and seed production.

Much variation was noted in number of seed per pod and number of pods per cluster. The poor seedling plants had very few of either while the best seedling plants had 10 to 12 seed per pod and 20 to 30 pods per cluster. This was reflected to some extent by the percentage of seed threshed from clusters harvested in the different states. Less than 10% by weight was secured in Virginia, approximately 10% in Alabama and Mississippi, and nearly 20% in Georgia, South Carolina, and North Carolina. Collections in Alabama in previous years had yielded about 18%.

The best condition for Kudzu seed production observed by the writer was old vines in small to medium sized trees in permanent pastures with deep topsoil. Presumably, the limit in area for continued growth, the constant pruning by grazing the lower leaves and overhanging vines, and a better than average moisture storage capacity in the soil provided a good environment for seed production.

The second best condition was in deep gullies with soil favorable for Kudzu growth and either trees, bushes, or steep sides on which the Kudzu could climb. Presumably, the gullies held down excessive vegetative growth and furnished ample moisture during dry periods of late summer and fall.

It is hoped research workers will determine more accurately the influences affecting Kudzu seed production and that plant breeders can develop better seed-producing strains. More seed are needed to replace the former importations from Japan.—PAUL TABOR, *Soil Conservation Service, Spartanburg, S. C.*

## BOOK REVIEW

## SOILS AND FERTILIZERS

*By Firman E. Bear. New York: John Wiley and Son, Inc. Ed. 3. XI+374 pages, illus. 1942.*

THIS is the third edition of the author's well-known text "Soil Management". The change in title was decided upon "because of the ever-increasing importance of lime and fertilizer materials as aids in maintaining and increasing the productivity of the soils and because the book deals, in considerable part, with these materials and their uses". The format has been changed, the number of pages reduced, but the over-all length of the text does not appear to be greatly altered. The 24 chapter headings remain the same, but the divisional headings used in the earlier editions have been omitted. To many who have used this text, this omission of divisional headings and even the change in title will probably seem questionable.

The author stresses throughout the soil as a medium for growing crops and the factors affecting its ability to produce and to continue to produce crops economically. He is not greatly concerned with a study of the soil as an independent academic discipline. The style is terse and to the point. Many beginning students will probably find the treatment of some topics too brief and, at times, a little vague and inconclusive.

The selection of subject matter is well suited to the needs of the average sophomore in a college of agriculture, especially that large majority who have only a general interest in the soil as a medium in which to grow crops.

There are some inconsistencies in the placement of sectional headings. For example, on page 29, we find the heading, "Water as an agent for regulating the temperature". In the first five paragraphs under this heading, osmotic pressure, wilting, "starter solutions", and plasmolysis are discussed. Temperature is briefly mentioned only in the last three sentences of the section.

Considerable new material has been introduced, for example, a couple of pages are devoted to the productivity index. Many of these introductions are very brief and fragmentary and may be hard for the student receiving his first introduction to these topics to grasp unless they are amplified in lecture, discussion period, or library.

We have pointed out what seems to us to be some of the shortcomings of the book. To the many who have found the earlier additions useful, this new edition will be especially welcome. (R. B.)

## AGRONOMIC AFFAIRS

### THE 1942 SPRAGG MEMORIAL LECTURES

DOCTOR H. M. Tysdal, Agronomist, Division of Forage Crops and Diseases, U. S. Dept. of Agriculture, and of the Department of Agronomy, College of Agriculture, University of Nebraska, Lincoln, Nebr., gave the eleventh in the series of Frank Azor Spragg Memorial Lectures at Michigan State College, March 10 to 13, 1942.

The memorial lecture proper dealt with "New Aspects of Forage Crop Improvement." The four other lectures centered around the topics "Controlled Testing as Aids to the Plant Breeder," "Polyploidy in Farm Crops and Inheritance in Tetraploids", "Factors Affecting Seed Production in Alfalfa", and "Alfalfa Breeding".

### WANTED—NEWS ABOUT AGRONOMISTS IN THE ARMED FORCES

NEWS items of interest to readers of the JOURNAL are always welcomed by the Editor and never more so than during the present emergency. We are especially desirous of learning about agronomists who join the armed forces. Also, items concerning war emergency projects are solicited where such information can be made public.

#### NEWS ITEMS

DOCTOR J. B. HESTER of the Campbell Soup Company Research Laboratory at Riverton, N. J., is now on foreign duty with the Army.

—A—

DOCTOR RALPH M. WEIHING of the Department of Agronomy, Colorado State College, Fort Collins, Colo., has been called to active duty as a captain with the Army and is now stationed at Fort Sill, Oklahoma.

—A—

OTTO H. COLEMAN has resigned his position as Assistant Agronomist at the Colorado Agricultural Experiment Station to accept a position with the Office of Sugar Investigations, U. S. Dept. of Agriculture. Mr. Coleman is now located at Meridian, Miss., where he is working with sorghums.

—A—

R. F. CHANDLER, JR., Charles Lathrop Pack Research Associate Professor of Forest Soils at Cornell University, recently returned from sabbatical leave. Professor Chandler visited forest soil experiment stations in California, the Pacific Northwest, British Columbia, Alaska, Mexico, and in the Gulf Coast states. Several months were spent in study at the University of California at Berkeley.

—A—

RALPH M. ARMS, Instructor in the Agronomy Department of South Dakota State College and Assistant in the South Dakota Agricultural Experiment Station, has been called to active military duty.

THE SUMMER meeting of the Southern Pasture and Forage Crop Improvement Conference scheduled to meet at the Florida Agricultural Experiment Station in July has been postponed. In all probability, the postponement will remain effective for the duration of the war.

—A—

THE SUMMER meeting of Section O of the American Association for the Advancement of Science scheduled to be held in Ann Arbor, Mich., the week of June 22 has been cancelled. In fact, the entire schedule of the A. A. A. S. for the summer has been dropped. Section O will hold its next regular meeting in New York City on December 28, 1942. Plans are underway for a program at that time dealing with some phase of nutrition.

### ERRATUM

IN the article by J. H. Hunter and R. D. Lewis on "Influence of Fertilizer and Time of Its Application on Growth, Yield, and Quality of Pecans", which appears in the February number of the JOURNAL, errors occur in Table 3, page 182, under the heading, "Source of Variance". The table should read as follows:

Source	DF	Sum of squares	Variance
Total .....	349	329,465.38	
Between years .....	4	234,897.58	58,724.40
Between treatments .....	6	6,477.70	1,079.62
Years $\times$ treatments .....	24	21,064.54	877.69
Error .....	315	67,025.56	212.78

SE of sum for a treatment for 1 year— $\sqrt{212.78 \times 10} = \sqrt{2127.8} = 46.12$

SE of a mean for a treatment for the 5-year period— $\sqrt{\frac{212.78}{50}} = \sqrt{4.256} = 2.06$

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### STRUCTURE OF SOME ORGANIC SOILS AND SOIL MIXTURES AS SHOWN BY MEANS OF $pF$ MOISTURE STUDIES<sup>1</sup>

VERNON C. JAMISON<sup>2</sup>

BECAUSE of their use as soil amendments in greenhouses, on lawns and flower beds, and in nurseries and orchards, organic soil materials have been studied with respect to their effects on the physical and chemical properties of mineral soils. Together with other physical effects observed it is often concluded that organic amendments not only increase the moisture-holding capacity, but also the supply of water available to growing plants.

Feustel and Byers (2)<sup>3</sup> found that some dry sedge and reed peats were capable of adsorbing about twice as much water (volume basis) as a certain clay loam. However, the peats and also the peat-soil mixtures retained about twice as much water at the "wilting point" as did the mineral soil. The peats and mixtures lost water more rapidly than a clay or a clay loam, beginning at saturation, but when the moisture content was low the mineral soils dried more rapidly. This indicates that the conductivity for the peat soils is lower at low moisture contents than for the mineral soils but is higher in the region near saturation. This conclusion is verified by the capillary conductivity measurements of Richards and Wilson (4) and also of Wilson and Richards (7). In the region of 200 to 300 cm of water tension, the woody peats studied by the latter authors were found to conduct water much more slowly than a loam and more slowly than a sand. Movement was so slow that time to establish equilibrium prohibited studies at higher tensions.

Field studies made by Andersen (1) are in harmony with these laboratory and greenhouse observations. He found for peat lands

<sup>1</sup>Condensed from a thesis presented by the author to the Graduate School at Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy conferred June 1941. Received for publication October 10, 1941.

<sup>2</sup>Citrus Experiment Station, Lake Alfred, Florida. The author wishes to express his gratitude to Dr. Richard Bradfield; to Dr. B. D. Wilson (deceased), who aroused the author's interest in the moisture tension relations of organic soils; and to fellow workers at Cornell and elsewhere for materials and helpful suggestions.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 403.

near Oswego, N. Y., that the roots of lettuce plants lowered the water table several inches in their immediate vicinity without affecting the level appreciably a few feet away.

Tukey and Brase (5) have conducted experiments designed to compare the effect of dry granulated and moist granulated peats mixed with mineral soil and mineral soil alone placed in tree holes with young trees at time of transplanting. They felt that the somewhat better root and shoot growth with peat plus soil over soil alone was due chiefly to better aeration. They concluded that since the roots were much more confined to the region of the tree hole with soil alone than with peat plus soil, the benefit was probably not a direct effect on the moisture supply. The fact that the differences between treated and check plants were greatest after summers of higher rainfall seems to indicate that aeration may have been the factor involved. Tukey and Brase did not offer an explanation for the somewhat better response with wet peat over that with dry peat. It is conceivable that the dry peat withdrew moisture from the adjacent mineral soil in the tree hole but did not increase in water content appreciably until after the young trees had suffered from drought for some time. However, this does not explain why the effect lasted for more than one season. It is more likely that since the wet peat was swelled, less of it was tamped into each tree hole. Upon wetting, the "dry peat" would have a lower porosity and root aeration would be more restricted.

Wilde and Hull (6) concluded that dry peat mulches due to their high moisture adsorption causes drought of nursery seedlings (Norway pine, *Pinus resinosa*, and Norway spruce, *Picea excelsa*). The high relative amount of "unavailable" water held by peat was doubtless an important factor involved. Even though the mineral soil was somewhat above the wilting region in moisture content before the dry peat application, both peat and soil were probably below their critical values after a few days. Evaporation would accentuate this effect, but assuming the loss to the atmosphere to be nil, it is still conceivable that droughty conditions would result.

In an earlier study (3), organic matter additions were observed to have rather interesting effects on the physical properties and the productivity of a Dunkirk silty clay loam. For comparison with these results and to obtain much-needed information about the structural nature of typical organic soils the studies reported in this paper were made.

## EXPERIMENTAL

### SOILS AND METHODS OF STUDY

As nearly as was feasible for the materials studied the same methods as previously outlined (3) were used. Unfortunately, some of the samples could not be taken from the field in an "undisturbed" state. The rather loose condition of some of the peats made this impossible.

Some of the characteristics of the samples used are shown in Table 1. The woody peats were put through a coarse sieve (5-mm)

to remove large pieces of wood, an effort being made not to crush lumps present in the rather loose material. The H layer of a mor came from a supply used for another purpose. It had been screened and mixed for uniformity. The mossy commercial peat (Cherry River) was used as taken from a bale in which it was shipped from Maine. It seemed quite uniform, though somewhat loose and coarse.

TABLE 1.—*Characteristics of some of the samples used.*

Soil	Source	Volume weights	True specific gravity	Air-dry moisture (approx. pF 6)	
				Dry weight basis	Volume basis
"Dry peat" . . .	Oswego, N. Y.	0.270	1.61	13.8	3.72
"Wet peat" . . .	Oswego, N. Y.	0.305	1.41	11.6	3.53
H layer of a mor. . . . .	Adirondacks of New York	0.230	1.34	8.60	1.98
Cherry River peat . . . . .	Maine	0.087	0.72	15.3	1.33
Dunkirk silty clay loam surface . . . . .	Caldwell Field, Ithaca, N. Y.	1.14	2.69	1.50	1.71
"Clay" . . . . .	Ithaca, N. Y.	1.41 $\pm$ .02	—	2.13	3.03

In the study of the clay and the peat-clay mixtures, the same technic was used as that with the Dunkirk soil samples (3). For the organic soils alone, the procedure was as follows: Loose peats were packed firmly into large 500-cc Jena sintered glass funnels, an effort being made to obtain the same state of packing for each soil. Each funnel, below the porous plate, was filled with water and the drawn-out stem was attached to a dropping funnel in the same manner already described for the small desiccators (3). Weighing was done by suspending the funnel beneath a balance in a weighed cord sling. The volume of the empty funnel was estimated within about 1 cc by leveling the bowl with a short spirit level and filling it with a weighed volume of water. The soil was wetted slowly by bringing the water surface even with the plate for 2 days. It was then flooded by raising the water level about 5 cm above the top of the soil. The excess peat that had swelled above the rim of the funnel was removed and a petri dish inverted over the funnel to prevent water loss by evaporation.

The system was carried through a series of equilibrations at different tensions in the same manner as with the inorganic soils (3). After the last equilibration and weighing, a moisture sample was taken and some of the peat was used for vapor pressure studies. The remainder furnished a supply for centrifuging. The vapor pressure and centrifugal technics, as well as calculations, were made in the way described for mineral soil (3).



COMPARISON OF WEIGHT AND VOLUME EXPRESSION  
OF MOISTURE CONTENT

The "dry" and the "wet" peat samples were taken from vegetable land near Oswego, N. Y. The terms "dry" and "wet" are used by farmers to characterize their physical properties. The dry peat shows marked resistance to wetting when air dried and probably has less sedge in it. The dry peat also shows greater swelling upon wetting. It is more colloidal in its properties as indicated by this characteristic and also its higher moisture content when air dried (Table 1).

In Fig. 1 the pF curves for these soils expressed on the dry weight basis are compared with a curve for a Dunkirk silty clay loam surface soil in a fair structural condition. For the same weight of soil, one of

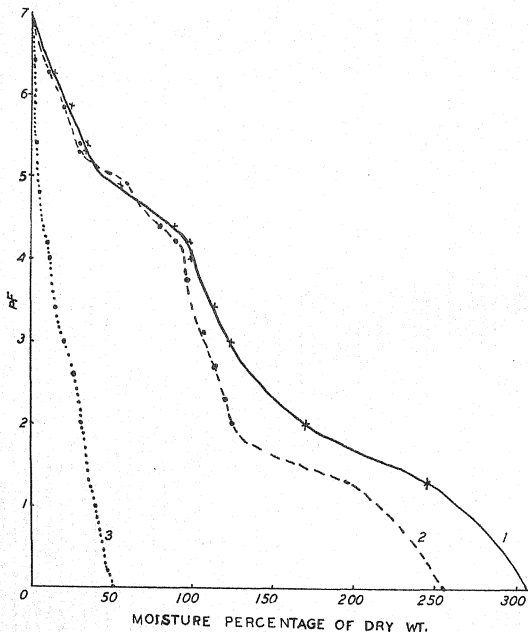


FIG. 1.—Relation between pF and moisture percentage of dry weight for two woody peat samples and a mineral soil sample. 1, "dry peat"; 2, "wet peat"; 3, Dunkirk silty clay loam surface soil.

these peats would provide much more pore space than the mineral soil, but a much greater volume of material would be involved. The moisture data for these same three soils are shown plotted on the volume basis in Fig. 2. The peats show a distinct advantage as far as total pore space is concerned, but as Feustel and Byers (2) found with a certain peat, the quantity of water held in an "unavailable" form is also great.

In a comparison of the peats and the mineral soil, it is to be noted the gentle flex in the pF curve for the Dunkirk soil comes at about 0.5 pF unit below those of the peats. The maximum frequency of pore sizes in the plant growth range below the wilting range (that is, below about pF 4.2) is not only greater but occurs at a higher pF for the peats than for the mineral soil.

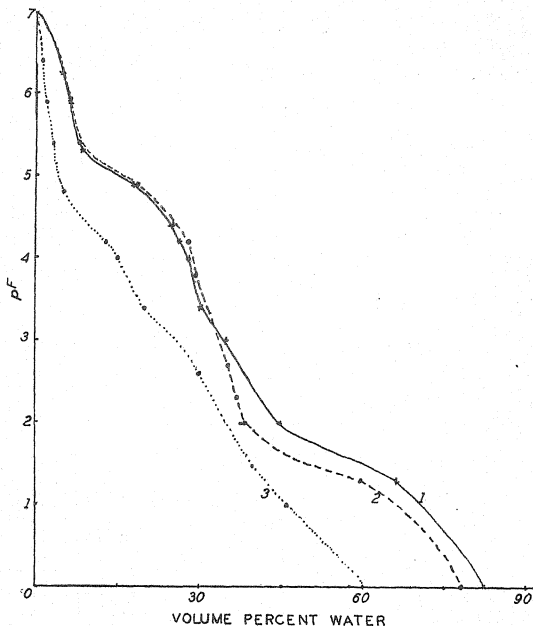


FIG. 2.—Relation between pF and volume percentage moisture for two woody peat samples and a mineral soil sample 1, "dry peat"; 2, "wet peat"; 3, Dunkirk silty clay loam surface soil.

The pore size distribution graphs for these three soil samples are shown in Fig. 3. The bimodal type of frequency is the noticeable feature of the woody peat samples. This is in distinct contrast to the weakly bimodal character of the distribution shown by the Dunkirk sample. The larger total pore space of the peat is made up chiefly of small macropores and ultra fine micropores. Only the former are of significance so far as moisture supply to plants is concerned. However, they are drained at relatively low tensions (about 100 cm water). These pores are of more importance in the provision of good aera-

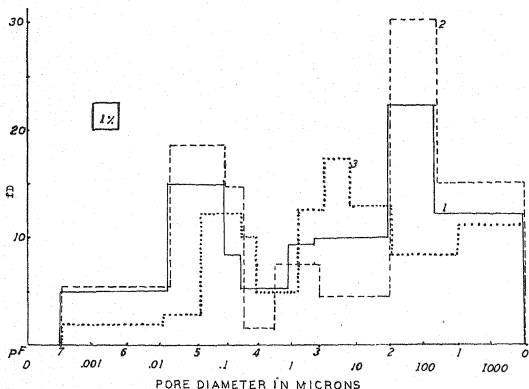


FIG. 3.—Pore size distribution for two woody peat samples and a mineral soil sample. 1, "dry peat"; 2, "wet peat"; 3, Dunkirk silty clay loam surface soil.

tion. The mineral soil is low in pores that are probably most closely related to permeability (3), but is rich in those which seem to be related to productivity in the Dunkirk soil, between  $pF$  2.7 and 4.2.

In consideration of the two different methods used in plotting moisture content, the dry weight value has the advantage that shrinkage does not affect the base of expression. It is not necessary to determine the volume of the soil and, for comparative purposes, this method is suitable with soils close to the same specific gravity. But in arriving at some notion of the effect that the mixing of soils for greenhouse purposes has on the volume and nature of pores, this procedure can easily be misleading. To have a strictly comparable base for soils, we should use something like a water-solid space ratio. This would differ from the volume percentage expression used in this paper only in being a ratio of liquid to solid space rather than liquid to total soil volume. The difference between these volume expressions

is not essential when the latter is based on the total volume at saturation (or the volume at some other equipotential level).

One weakness of the volume expression used here is that the general slope of a  $pF$  curve depends, in part, upon the moisture content found at saturation which is not very reproducible. This is not as serious as it may seem, however, since an error in the measured volume amounting to more than 2% moisture was never found by calculating the total volume from the determined specific gravity and saturation moisture content. The inaccuracy as far as the base is concerned is spread out over the whole  $pF$  scale.

#### EFFECT OF PEAT ON POROSITY OF MINERAL SOILS

The Cherry River peat studied was a commercial sedge peat from Maine. The peat-clay mixture and clay samples were taken from experimental rose bed plots used by the Floriculture Department. The H layer of a mor, included here for comparison with the peat, was taken from over a Beckett fine sandy loam in the Adirondacks of New York.

The  $pF$  curves for the two organic soils are shown in Fig. 4. Both of these soils are different from the woody peats in holding smaller quantities of water at the "wilting point" ( $pF$  4.2). The commercial peat sample is especially noticeable in this respect. Another interesting feature of the Cherry River peat is the broad plateau effect in its curve between  $pF$  1.3 and 2.0. The other organic soil samples exhibit this feature, but to a lesser degree.

The distribution of pore space for these samples is shown in Fig. 5. The greatest pore size frequency is in a fraction including the smaller macropores and larger micropores. They are quite different from the woody peats in having a relatively small proportion of their total porosity in the "unavailable" range. But the volume percentages of pores in both these fractions in the mineral soil are much lower than for any of the organic soil samples studied.

In plots from which the clay-peat mixtures and also the clay soil samples were taken, a clay soil was mixed in a gross way and used to prepare the "clay" rose bed. Beds were also prepared by mixing this clay in a roughly 1:1 ratio by volume with the peat. Four samples were taken from these beds, and two samples of the clay were used. The mean  $pF$  curve for the clay-peat samples is shown in Fig. 6. The average of the two clay samples and the curve for the peat are superimposed on the graph. The clay curves are somewhat like those of the Dunkirk subsurface samples (3). They differ in that much more moisture is held over the "vapor pressure" range,  $pF$  4.2 to 7.0, indicating a higher content of colloidal clay.

The effect of the peat on the heavy, dense clay is interesting. Both the total porosity and the porosity in the approximate plant growth range, below  $pF$  4.2, is increased. The amount added is not practical except in greenhouses, and then the mineral soil ordinarily used would have more desirable physical properties than this clay. Contrast the effect here with that shown in Fig. 7 where Port Byron peat was added to a Dunkirk silty clay loam in somewhat more than

moderate amounts (3). In either case, the dominant change is in the pF range near the pF flex of the soils so treated and that of the peat added. That is, compare curves 2 and 3 in Fig. 6. The slopes are simi-

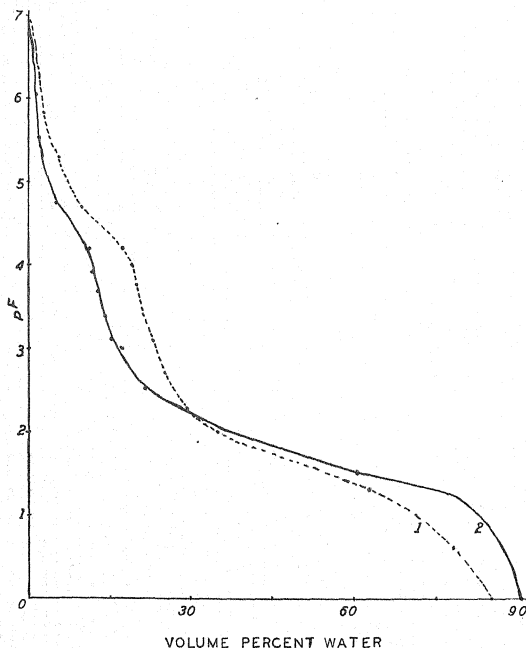


FIG. 4.—Relation between pF and volume percentage moisture for two different organic soils. 1, H layer of a mor; 2, Cherry River peat.

lar above pF 3 and below pF 1, but between these two potential levels they are quite different.

With a soil like the Dunkirk silty clay loam in rather poor structure, the addition of moderately great amounts of peat had little favorable effect on porosity. However, with the addition of some peats to heavy dense clays, it is possible to get an improved condition

in the moisture-supplying power of the soil. In any case, the addition of the peats studied can be expected to increase soil aeration for most finer textured mineral soils.

In some respects, the comparison of the porosity of organic and mineral soils is not quite fair to the former. In their natural environment, most peats have a rather high water table. The soil is relatively loose, and in general, plant roots tend to permeate it and encompass comparatively large volumes so that the effective supplying power of the soil to the growing crop is doubtless enhanced. That is, the

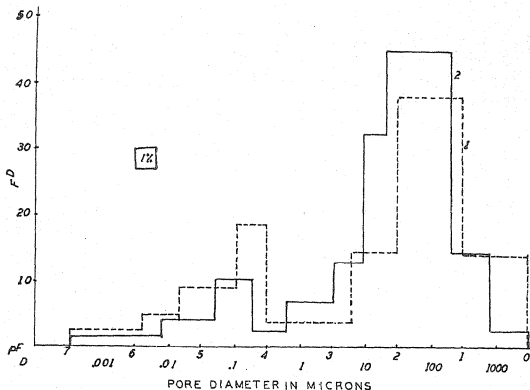


FIG. 5.—Pore size distribution of two organic soils. 1, H layer of a mor; 2, Cherry River peat.

conductivity of peats is so low when they are still at rather low moisture tensions that practically no water flows to the roots, but the roots may grow into wetter zones sufficiently rapidly to supply the plants with moisture.

Though the H layer of a mor compares unfavorably with a mineral soil of moderately good structure with respect to field moisture supply, the picture is incomplete. The rainfall in the natural environment of this soil is usually higher and more favorably distributed than with many productive mineral soils. To speak of the "unavailable" water in a soil in such an environment may be very misleading.

On the other hand, when stripped of vegetation and when periods of drought occur, many of these organic soils dry out readily and are severely eroded by the wind. The similarity of the curve for the Cherry River peat to that of a sand fraction is enlightening.

## CONCLUSIONS

In pore size distribution studies, the volume basis of moisture expression is more useful and gives a better picture of structure than the commonly used dry weight basis.

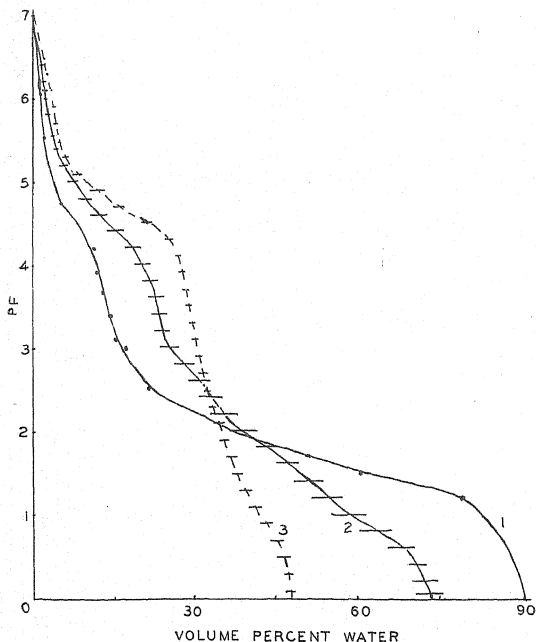


FIG. 6.—Mean pF moisture curves for clay and peat-clay mixture samples compared with peat used in the mixture. Lines parallel to base indicate standard errors. 1, Cherry River peat; 2, clay-peat mixture; 3, clay alone.

The organic soils studied, though widely different in many properties, all showed the greatest frequency of pores between pF 1.0 and 3.0 with a smaller second maximum between pF 4 and 5. The greatest change in pF moisture relations of mineral soils when peats are added seems to be in the lower of these two ranges. Some change is

effected in the pF 4 to 5 range, with the least noticeable shifts in the intermediate "field moisture" range.

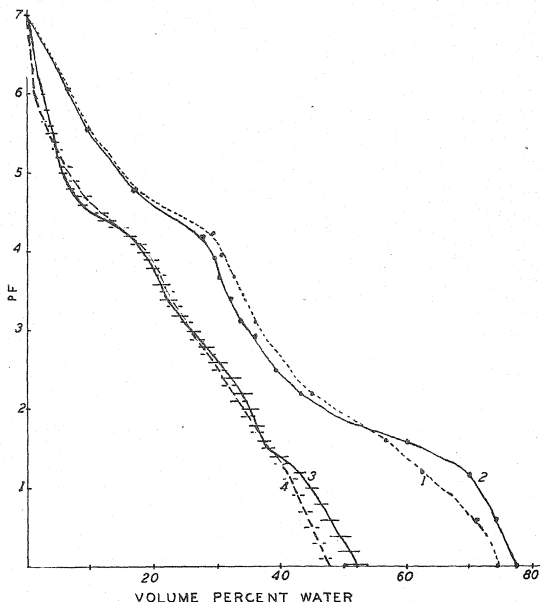


FIG. 7.—The residual effect of Port Byron peat. 48.9 tons of peat in 1935 and 42.8 tons in 1936 were added per acre. Soil samples taken June 1, 1940. 1, stored peat (air-dried); 2, moist peat (leached); 3, peat-soil plot samples; 4, south seventh year cultivated series, Dunkirk silty clay loam, no peat added.

The lower part of the pF curves of organic soils studied resemble that of a sand fraction in shape. The low conductivity and wind erosion of peats when still at relatively low tensions can be explained from the nature of their moisture relationships.

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**COTTON WILT IN ALABAMA AS AFFECTED BY POTASH  
SUPPLEMENTS AND AS RELATED TO VARIETAL  
BEHAVIOR AND OTHER IMPORTANT  
AGRONOMIC PROBLEMS<sup>1</sup>**

H. B. TISDALE AND J. B. DICK<sup>2</sup>

COTTON wilt, a soil-borne disease caused by *Fusarium vasinfectum* Atk., has been spreading gradually to new areas since it was discovered by Atkinson in 1892. Although damage from wilt has been minimized by growing wilt-resistant varieties, the authors do not know of any case where the disease has been completely eradicated. Hence, the breeding of cotton for wilt resistance will be a specialized problem for years to come.

One major difficulty confronting the breeder is the lack of specific and comprehensive information regarding the causes of the peculiar behavior of the cotton wilt organism in relation to its environment. It is known that such factors as seasons, fertilizers, and varieties of cotton affect the development of wilt symptoms. It is believed by many cotton growers that the severity of wilt damage is increased in cotton following such crops as okra, sweet potatoes, cucumbers, and cowpeas. Some workers have observed that a variety of cotton may be quite resistant to wilt at one location and comparatively susceptible at another location. This has recently led to some speculation as to the possibility of the existence of highly specialized physiologic strains of the wilt organism.

A practical method for developing and maintaining a thoroughly and uniformly wilt-infested soil area to facilitate breeding cotton for wilt resistance might be evolved if the causes of these confusing manifestations of the wilt disease were known. Some workers (3, 5, 8, 9, 10)<sup>3</sup> have reported promising results from the use of cultures of the wilt organism in the inoculation of soil for testing the differential response of varieties of cotton to the wilt disease. These indicate varying success and serve to emphasize the need for fundamental investigations relative to the nature of wilt resistance in cotton and to the interaction of hereditary and environmental factors.

A number of investigators (2, 3, 4, 9, 12) agree that cotton wilt can be controlled by the use of suitable varieties together with the use of fertilizers containing adequate amounts of potash. Prior to the

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<sup>2</sup>Associate Plant Breeder and Associate Agronomist, respectively. Grateful acknowledgment is made to H. D. Barker, Principal Pathologist, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, for plans for the regional wilt experiments, for analyzing the data, and for helpful suggestions concerning the work herein reported.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 425.

initiation of the studies herein reported the importance of potash in relation to the development of wilt had been adequately demonstrated for the Mississippi Valley region only. Although other workers (1) have shown a differential response of other crops to nutritional deficiencies, similar behavior for cotton has not been conclusively demonstrated. The factorial design, which appears to have been used for the first time for field experiment with cotton wilt when the regional wilt experiments (two of which are included in this report) and related experiments (7) were organized, has facilitated the study of the differential response of varieties of cotton to varying amounts of potash and to the development of wilt.

The purpose of this paper is to report (a) the effect of applications of various amounts of potash on different types of wilt-infested soils on yields of cotton and the development of wilt in resistant, tolerant, and susceptible varieties of cotton under field conditions; (b) the effect of seasons, locations, and other factors on the development of wilt and the behavior of wilt-resistant varieties in Alabama; and (c) the field evidence for the existence or nonexistence of different physiological strains of the wilt organism in Alabama.

#### GENERAL EXPERIMENTAL PROCEDURE

Nitrate of soda, 16% superphosphate, and muriate of potash were used as the respective sources of N, P, and K in all experiments. The seed for all tests were treated with Ceresan. The cotton was grown by the cultural methods commonly used in the localities where the tests were conducted. Counts of wilt-affected plants (diseased and dead) were made at 20- to 30-day intervals during the growing seasons and the dead plants were removed at each count. The first counts were made as soon as possible after the plants had become established, and the final counts were made after the cotton began to open. The percentages of wilt-affected plants given in the Tables 1 to 4 represent the total number of plants killed by wilt plus the number of living plants showing distinct symptoms of the wilt disease at "midseason" counts, roughly the maximum blooming period. Table 5 is based on final counts.

Other experimental methods peculiar to any experiment or group of experiments here reported are given in the description of the experiments.

#### POTASH-VARIETY-WILT TESTS AT AUBURN, 1936-38

In 1935 a wilt-resistant variety of cotton was planted in a field of Norfolk loamy sand on which lime had been added and on which a heavy crop of a mixture of Austrian peas and vetch had been turned under. Although fertilizer of the usual N-P-K ratio was applied at the rate which previous experience had indicated was adequate, the cotton began to shed its leaves at midseason. At the end of the growing season defoliation was practically complete and there were many immature bolls which failed to open. A similar condition was observed in a number of localities in Alabama during the season of 1935 in fields of cotton following winter legumes.

This unusual condition was so widespread that an experiment was planned to study the influence of varying amounts of potash on its

development in connection with wilt, particularly in wilt-resistant varieties and strains of cotton. The affected area at Auburn was planted to Austrian peas and vetch in the fall of 1935 and a heavy crop was turned under in the spring of 1936. The experiment, consisting of four blocks of 33 100-foot, single-row plots, was set up on this area. A 2-8-0, 2-8-4, 2-8-8, and 2-8-16 fertilizer was applied to each of the four blocks, respectively, at the rate of 600 pounds per acre. Additional nitrogen was supplied by the turned-under legumes. Twenty-four varieties and strains of cotton, supposedly wilt-resistant, were planted on each block, systematically arranged, with a susceptible variety planted every fourth row as a check.

The results obtained in 1936 strongly suggested a difference in varietal requirement of potash for reducing wilt and increasing yield, although it was difficult to evaluate varietal interaction since the limited area available precluded replication of varieties within potash blocks. In 1937 the number of varieties was reduced to 11, permitting three replicates of each variety within each potash treatment in 1937 and 1938. Ten of the original varieties were used and one new variety, Farm Relief, was added. These 11 varieties represented five groups with respect to susceptibility to wilt, namely, resistant, highly tolerant, weakly tolerant, susceptible, and highly susceptible, as classified from the results obtained in 1936. They were randomized each year within three blocks for each potash treatment, using 100-foot, single-row plots. Wilt records and yield data were obtained as described under experimental procedure.

The results obtained from this experiment are summarized in Tables 1 and 2 and Figs. 1 and 2. The results for 1936 are also included in Table 5. While the potash-treatment effects were not measured with great precision due to the systematic split-plot arrangement for sub-blocks, the variations on land that had proved to be reasonably uniform for cotton production are so striking and the appearance of the plants were so characteristic of potash effects on cotton that the interpretation of the results for 1937 and 1938 are clear cut despite the experimental design. The influence of potash treatments on the development of wilt was more pronounced as the season advanced, but the potash-deficiency symptoms became so severe in the no-potash block that the detection of wilt symptoms became increasingly difficult. Therefore, due to the greater unreliability of the data for final count made in early fall, it seemed preferable to present the data for midseason count only. The analyses of variance for mid-season count and for yield are given in Table 2. In this table it will be noted that the error term was partitioned, as suggested by Tharp, Wade, and Barker (6) for heterogeneous data of this type.

These tables and graphs present definite evidence of a relatively constant ranking for varieties with respect to resistance regardless of seasonal variations or the over-all effects of potash treatment on wilt development. They also show (Table 2 and Fig. 1) significant differences in varietal response to the different levels of potash in both incidence of wilt and yield of seed cotton. Since certain conclusions drawn from this experiment are similar to those derived from the potash-variety studies at Moundville and Alexandria, some repetition

can be avoided by comparing the results from the three experiments. Hence, further details as to the interpretations are given in the discussion which follows the presentation of the results obtained from potash-variety-wilt studies at Alexandria and Moundville.

TABLE 1.—Mean effects obtained for three years, 1936-38, on Norfolk loamy sand at Auburn, Ala., on incidence of wilt and yield of seed cotton for 11 varieties and four rates of potash application.

	Wilt group*	% wilt-affected plants at midseason count for				Yield in lbs. per acre of seed cotton for			
		1936	1937	1938	3 yrs.	1936	1937	1938	3 yrs.

Means for Varieties by Seasons

Cook 307									
(B. Rhyne)....	R	1.4	3.2	1.5	2.0	811	993	902	902
Cook Wiregrass....	R	1.3	3.1	1.5	2.0	809	894	961	888
Clelewilt 6									
(Coker's).....	R	1.4	3.7	3.4	2.8	554	844	877	758
Dixie 14-5.....	HT	3.3	2.9	2.4	2.9	632	843	1,082	852
Dixie Triumph 85.	HT	3.8	4.1	2.0	3.3	819	791	1,080	897
Cook 144-68.....	HT	6.9	3.1	1.7	3.9	934	1,012	1,120	1,022
Cook 1006.....	HT	7.4	5.6	2.1	5.0	853	976	1,137	1,005
Missdel WR.....	WT	14.6	7.5	5.5	9.2	598	610	709	639
Miller 610.....	WT	14.7	10.0	4.6	9.8	931	955	1,167	1,018
Farm Relief.....	S	—	20.4	18.3	—	—	627	844	—
Half & Half.....	HS	67.5	44.3	40.9	50.9	513	556	781	617
Seasons†.....	—	12.2	8.8	6.6	—	745	847	987	—

Means for K Treatment by Season‡

K <sub>2</sub> O at 0%.....	20.7	12.9	12.5	15.4	656	496	596	583
K <sub>2</sub> O at 4%.....	11.6	8.1	4.9	8.2	872	955	1,211	1,013
K <sub>2</sub> O at 8%.....	9.4	7.4	5.5	7.4	822	976	1,141	980
K <sub>2</sub> O at 16%.....	7.5	6.5	3.4	5.8	631	964	1,000	865

Differences Required for Significance (Odds 99:1) Between Means

Varieties (1)†.....	—	3.3	2.0	—	—	102	153	—
Varieties (2)§.....	—	17.3	15.9	—	—	85	198	—
K treatment.....	—	9.0	6.9	—	—	199	244	—

For means for K treatments by varieties, grouped according to highly significant differences for reaction to wilt, see Figs. 1 and 2.

\*"Wilt Group" represents the classification for wilt resistance on the basis of 1936 observations in Alabama tests. The symbols R, HT, WT, S, and HS signify resistant, highly tolerant, weakly tolerant, susceptible, and highly susceptible, respectively.

†Farm Relief was omitted in the calculation of means for seasons and for K<sub>2</sub>O by seasons because it was not grown in 1936.

‡Comparisons between the nine resistant (R), highly tolerant (HT), and weakly tolerant (WT) varieties.

§Comparisons involving the susceptible (S) and highly susceptible (HS) varieties.

POTASH-WILT TESTS AT MOUNDVILLE AND ALEXANDRIA

1937-39

In 1937, the Division of Cotton and Other Fiber Crops and Diseases of the Bureau of Plant Industry, U. S. Dept. of Agriculture, cooperat-

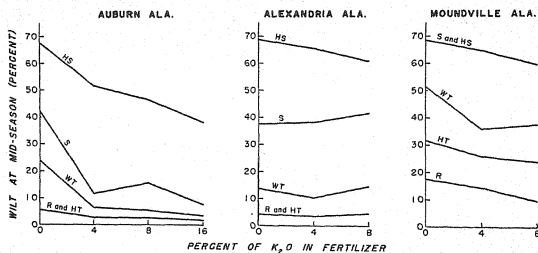


FIG. 1.—Interaction of varieties, grouped according to reaction to wilt, by potash treatments at three locations in Alabama as measured by the percentage of wilt at midseason (late July-early August counts) for 3 years. These graphs were obtained by restratification of the data given in Tables 1, 3, and 4. The symbols R, HT, WT, S, and HS signify resistant, highly tolerant, weakly tolerant, susceptible, and highly susceptible, respectively, as classified under "Wilt Group" in the above-mentioned tables.

ing with the states in the Cotton Belt, planned an experiment to study the effects of varieties of cotton and applications of potash in relation to the cotton wilt disease. This experiment was conducted at two locations in Alabama on wilt-infested soils; one near Moundville on Ochlockonee sandy loam (deep phase), and the other near Alexandria on Decatur clay loam. The incidence of wilt in all varieties in 1936 at these two locations is included in Table 5, and on the basis

TABLE 2.—Summary of analysis of variance for wilt count at midseason and yield of seed cotton from potash-variety test at Auburn, Ala., 1937-38.

Source of variance	D.F.	Mean squares for			
		Wilt at midseason for		Yield of seed cotton for	
		1937	1938	1937	1938
Blocks.....	2	539.36	265.77	23.0810	22.5324
K treatments.....	3	587.71*	780.04**	117.9972**	175.7833**
Error (a).....	6	96.71	57.64	3.0897	4.6309
Variety (1)†.....	8	72.14**	24.77**	12.4857**	19.4201**
V (1) × K.....	24	15.32	8.93**	0.9100	1.1048
Error (b).....	64	9.26	3.21	0.5730	1.2792
Variety (2)‡.....	2	9170.96**	8591.89**	53.6645**	25.2914**
V (2) × K.....	6	347.39	749.99**	1.7730**	6.5576*
Error (c).....	16	209.51	177.54	0.3272	1.7688

\*"P" values significant (odds 19:1).

\*\*"P" values highly significant (odds 99:1).

†Comparisons between the nine resistant (R), highly tolerant (HT), and weakly tolerant (WT) varieties.

‡Comparisons involving the susceptible (S) and highly susceptible (HS) varieties.

of these tests the two locations were chosen for the regional potash-variety-wilt studies. Moundville was selected because of the unusual severity of the disease in resistant varieties, and Alexandria was chosen because of severe wilting in the susceptible checks with very little wilt in the other varieties. In addition, Alexandria represents a reddish clay loam overlying limestone, classified as Decatur clay loam, and a region as well as a soil type in which wilt is not usually prevalent. These two tests were conducted for 3 years, 1937-39, on the same respective plots and each received like treatments, except that a fair growth of vetch was turned under preceding the cotton at Alexandria in 1937.

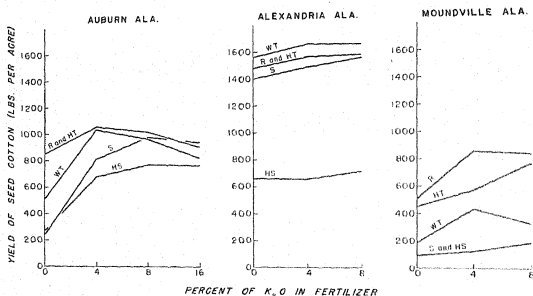


FIG. 2.—Interaction of varieties, grouped according to reaction to wilt, by potash treatments at three locations in Alabama as measured by the yield of seed cotton for 3 years. These graphs were obtained by restratification of the data given in Tables 1, 3, and 4. The symbols R, HT, WT, S, and HS signify resistant, highly tolerant, weakly tolerant, susceptible, and highly susceptible, respectively, as classified under "Wilt-Group" in the above-mentioned tables.

The experiment was arranged according to a factorial design, using three series of 36 single-row plots 100 feet long. Each year 12 varieties of cotton representing the resistant, highly tolerant, weakly tolerant, and susceptible groups were used. Eight of these varieties were continued at both locations for the 3 years. Certain substitutions, [as shown in Tables 3 and 4, raised the total number of varieties or strains included in the experiment to 17. (See Fig. 3.)

Three fertilizer treatments, 6-8-0, 6-8-4, and 6-8-8, were applied at the rate of 600 pounds per acre. Fertilizer treatments were randomized within series the first year (1937), after which each plot received the same fertilizer treatment as it did in 1937. Varieties were randomized within series each year so that each variety received a 6-8-0, a 6-8-4, and a 6-8-8 treatment in each series every year. All the mineral fertilizers and  $\frac{1}{2}$  of the nitrogen were applied before planting. The remaining  $\frac{1}{2}$  of the nitrogen was applied as a side-dressing as soon as possible after the cotton was thinned.

The incidence of wilt and yields of seed cotton were obtained for each plot and the averages computed for each location. Two errors occurred during the course of the experiment at Moundville. In the early fall of 1937, a light picking was made on all plots which was not weighed. This light picking was considered to have little effect on the final results and the remaining yields were recorded for 1937. In 1939 a little over  $\frac{1}{2}$  of the area comprising the plots was fertilized at the rate of about 300 pounds of a 6-8-4 fertilizer per acre. Regardless of this error that equally affected all blocks, the fertilizers were applied according to the plans and the experiment was completed for 1939.

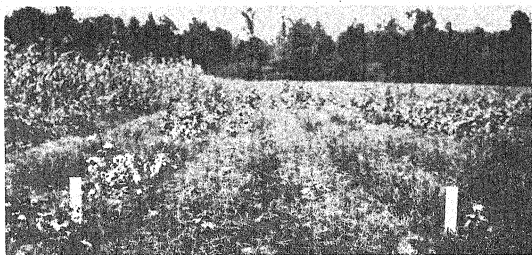


FIG. 3.—Showing Dixie Triumph 85, highly tolerant, on the two staked rows; *left*, fertilized with 600 pounds of 6-8-4; *right*, fertilized with 600 pounds of 6-8-0. Two rows in center are fertilized with 600 pounds of 6-8-0; *left*, Cook 144-68, highly tolerant; *right*, Deltapine 11 A, weakly tolerant. Note the severity of the disease here as shown by the few plants that have survived and by the development of crab-grass in certain rows. The crab-grass growth indicates lack of competition from the cotton plants after "laying-by" time. Moundville, 1938.

In spite of these unfortunate errors at Moundville, it is felt that the data are valuable. Results for 3 years, 1937-39, at both locations are given in Tables 3 and 4 and in Figs. 1 and 2. Further discussion concerning these locations and the interpretation of experimental results are given in the comparison of results of the potash-variety-wilt tests at Auburn, Moundville, and Alexandria following the presentation of Figs. 1 and 2.

#### COMPARISON OF RESULTS OF THE POTASH-VARIETY-WILT STUDIES AT AUBURN, MOUNDVILLE, AND ALEXANDRIA, ALA.

In considering the results obtained for 3 years at three widely separated locations in Alabama it should be borne in mind that the choice of these three locations was fortunate from the standpoint of sampling areas which gave marked contrast for conditions under which wilt occurs in Alabama. Auburn may be considered to repre-



TABLE 3.—Mean effects obtained for 3 years, 1937-39, on Ochlockonee sandy loam at Moundville, Ala., on incidence of wilt and yield of seed cotton for 17 varieties and three rates of potash application.

	Wilt group*	% wilt-affected plants at midseason count for				Yield in lbs. per acre of seed cotton for			
		1937	1938	1939	3 yrs.†	1937	1938	1939	3 yrs.†
Means for Varieties by Seasons									
Sea Island.....	I	0.0	—	—	0.0	545	—	—	433
Cook 307.....	R	10.7	11.9	11.0	11.2	1,022	863	595	827
Dixie Triumph 12.	R	8.0	18.6	14.8	13.8	859	670	687	739
Clelewilt 6 (Coker's).....	R	14.6	11.6	—	14.5	898	1,011	—	901
Clelewilt 7 (Coker's).....	R	—	—	18.9	16.0	—	—	371	457
Sikes WR Staple..	HT	18.2	—	—	21.2	761	—	—	604
Cook 144.....	HT	16.4	20.0	27.6	21.3	698	407	519	541
Coker's 4 in 1....	HT	—	—	26.9	22.7	—	—	668	823
Dixie Triumph 85.	HT	26.5	25.2	33.1	28.3	642	653	418	571
Toole.....	HT	24.6	35.8	—	32.9	729	434	—	524
Dixie 14-5.....	HT	19.9	40.2	47.1	35.7	786	356	362	501
Deltapine 12.....	WT	—	—	48.2	40.8	—	—	172	212
Deltapine A.....	WT	—	39.2	—	40.8	—	290	—	312
Rowden 2088.....	WT	—	34.2	55.4	41.3	—	525	233	427
Miller 610.....	WT	35.0	39.1	57.1	43.7	285	350	311	315
Coker 100.....	S	56.4	59.2	65.2	60.2	230	112	57	133
Half & Half.....	HS	70.3	56.8	78.1	68.4	219	82	108	136
Seasons†		30.4	33.9	41.8	—	593	437	382	—

## Means for K Treatment by Seasons§

K <sub>2</sub> O at 0%.....	27.0	39.5	47.1	37.9	450	350	265	355
K <sub>2</sub> O at 4%.....	24.9	30.7	39.0	31.5	679	529	373	527
K <sub>2</sub> O at 8%.....	23.2	27.7	34.8	28.6	789	559	487	612

## Differences Required for Significance (Odds 99:1) Between Means\*\*

Varieties.....	9.1	10.7	13.8	—	††	251	329	—
K treatments.....	4.6	5.3	6.9	—	—	126	164	—

For means for K treatments by varieties, grouped according to highly significant differences for reaction to wilt, see Figs. 1 and 2.

\*"Wilt Group" represents the classification for wilt resistance on the basis of performance in 1936 and subsequent tests in Alabama. The symbols I, R, HT, WT, S, and HS signify immune, resistant, highly tolerant, weakly tolerant, susceptible, and highly susceptible, respectively.

†Actual means are used for the eight varieties which were grown all three years. Weighted means were used for varieties grown one or two years only, in order to provide an approximate comparison with those grown for three years.

‡In order to judge seasonal effects, the means for seasons include only the eight varieties which were grown for all three years.

§Actual means for the 12 varieties grown each season.

\*\*Differences required for significance were calculated on the basis of the pooled error (70 D.F.). Alternative analyses with partitioned errors indicated that the pooled error was reasonably satisfactory for interpreting potash treatments and varietal behavior at Moundville.

††Not analyzed statistically due to early light picking which was not recorded and which might result in biased conclusions.

sent the sandy coastal plain area, the vast region in which wilt is most likely to be a serious agronomic problem in Alabama. Moundville is somewhat representative of the bottom land along small creeks and rivers where wilt occurrence is usually localized in sandy portions of

TABLE 4.—Mean effects obtained for 3 years, 1937-39, on Decatur clay loam at Alexandria, Ala., on incidence of wilt and yield of seed cotton for 17 varieties and three rates of polish application.

	Wilt group†	% wilt-affected plants at midseason count for				Yield in lbs. per acre of seed cotton for			
		1937	1938	1939	3 yrs.‡	1937	1938	1939	3 yrs.‡
Means for Varieties by Seasons									
Sea Island*.....	I	0.0	—	—	0.0	377	—	—	268
Cook 307.....	R	5.5	1.5	3.5	3.5	1,923	752	1,128	1,267
Dixie Triumph 12.....	R	1.6	1.0	3.4	2.0	2,356	1,339	1,335	1,677
Clelewilt 6 (Coker's).....	R	4.9	1.6	—	3.9	1,901	1,012	—	1,389
Clelewilt 7.....	R	—	—	5.5	4.1	—	—	1,513	1,706
Sikes WR Staple.....	HT	3.2	—	—	3.9	2,339	—	—	1,665
Cook 144.....	HT	2.3	2.8	9.4	4.8	2,178	1,101	1,464	1,581
Coker's 4 in 1.....	HT	—	—	5.9	4.4	—	—	1,739	1,961
Dixie Triumph 85.....	HT	1.9	2.2	7.1	3.7	2,346	1,175	1,312	1,611
Toole.....	HT	5.1	2.8	—	4.4	1,952	826	—	1,278
Dixie 14-5.....	HT	6.9	4.6	10.3	7.2	2,004	768	1,175	1,316
Deltapine 12.....	WT	—	—	19.2	14.4	—	—	1,494	1,685
Deltapine A.....	WT	—	12.3	—	14.7	—	1,032	—	1,455
Rowden 2088.....	WT	—	7.8	14.6	10.1	—	1,141	1,546	1,676
Miller 610.....	WT	9.3	7.2	20.4	12.3	2,176	1,175	1,670	1,674
Coker 100.....	S	31.5	28.2	57.0	38.9	1,927	1,227	1,283	1,479
Half & Half.....	HS	55.3	67.1	72.5	65.0	926	463	636	675
Seasons§.....		14.3	14.3	23.0	—	1,979	1,000	1,250	—
Means for K Treatment by Seasons**									
K <sub>2</sub> O at 0%.....		11.3	11.2	19.2	13.9	1,775	976	1,337	1,363
K <sub>2</sub> O at 4%.....		9.4	12.2	17.8	13.1	1,930	1,008	1,352	1,430
K <sub>2</sub> O at 8%.....		11.3	11.3	20.2	14.3	1,896	1,021	1,384	1,434
Differences Required for Significance (Odds 99:1) Between Means††									
Varieties.....		6.1	5.3	8.3	—	326	199	273	—
K treatment.....		3.1	2.7	4.2	—	163	100	136	—

For means for K treatments by varieties, grouped according to highly significant differences for reaction to wilt, see Figs. 1 and 2.

\*Varieties listed following the order of wilt resistance at Moundville.

†"Wilt Group" represents the classification for wilt resistance on the basis of performance in 1936 and subsequent tests in Alabama. The symbols I, R, HT, WT, S, and HS signify immune, resistant, highly tolerant, weakly tolerant, susceptible, and highly susceptible, respectively.

‡Actual means are used for the eight varieties which were grown all three years. Weighted means were used for varieties grown one or two years only, in order to provide an approximate comparison with those grown for three years.

§In order to judge seasonal effects, the means for seasons include only the eight varieties which were grown for all three years.

\*\*Actual means for the 12 varieties grown each season.

††Interaction V×K gave a significant (odds 10:1) "F" value for final wilt count in 1938. Differences required for significance were calculated on the basis of the pooled error (70 D.F.). Alternative analyses with partitioned errors showed that at Alexandria as at Auburn (Table 2) the error terms for comparisons involving the susceptible and highly susceptible varieties were very much larger than those for comparisons involving the tolerant-resistant varieties. Since the discussions and interpretations in this paper are substantiated by the graphical representations in Figs. 1 and 2, which agree with interpretations from the partitioned errors, and since it is proposed to publish the alternative analyses in a subsequent paper dealing with the regional wilt-variety studies, it was decided to omit the presentation of the analysis of variance for the Moundville and Alexandria studies.

the field, but in which wilt control is frequently of great local importance due to the severity of the disease. Moundville also repre-

sents the most severe development of wilt which has been encountered in Alabama. Alexandria represents the Piedmont or northern portion of the state where wilt is less likely to be a serious problem. On reddish clay loams overlying limestone it is unusual to find wilt so uniformly distributed as it was found in the field where the Alexandria test was located.

The results presented in Tables 1 to 4 and in Figs. 1 and 2 show several very interesting comparisons. While the various factors which these studies show to influence wilt development are not independent of each other, it may be advantageous to consider certain of the more striking main effects and interactions as measured by the amount of wilt which had developed at midseason and as measured by the yield of seed cotton. In attempting to discuss these factors in some logical sequence and in order to orient the reader as to the comparisons under discussion, the following marginal headings are used.

*Locations.*—Despite the various contrasts with respect to regions, soil types, and other factors represented by the three locations, Fig. 1 shows these locations were surprisingly uniform with respect to the amount of wilt in the susceptible check variety (Half & Half) at midseason, particularly for the no-potash treatment. From Tables 1, 3, and 4, however, it will be noted that the average amount of wilt for all varieties was very different, with Auburn averaging about 9%, Alexandria 17%, and Moundville 35%. No satisfactory explanation was obtained for the unusual severity of wilt at Moundville, especially on resistant varieties. The abundance of root knot nematodes and the amount of damage which they caused to the roots of the cotton plants may have been a contributing factor.

At harvest time in 1937 numerous plants from all varieties were examined in an attempt to establish an association of varietal differences with respect to nematode susceptibility and wilt development. Galls were found to be numerous and well-developed on the roots of all varieties and no clear-cut distinction with respect to nematode susceptibility was evidenced, with the exception that the immune Sea Island (Seabrook) was definitely more susceptible to root knot, as judged by the number and size of galls produced, than were the upland varieties.

From the standpoint of yields Alexandria was outstanding, all treatments and varieties averaging over 1,400 pounds of seed cotton for the 3 years, whereas at Moundville they barely averaged 500 pounds of seed cotton per acre. The yields at Auburn were intermediate, averaging 860 pounds. The growth of corn in adjacent plots, as well as the appearance of the soil, indicated that the soil at Moundville was fairly productive. It is believed that the severity of the disease, even in the most highly resistant varieties, was responsible for the low yields obtained at Moundville. This is indicated by the inverse relationship of the wilt and yield curves for Moundville (Figs. 1 and 2).

*Seasons.*—Due to the distance which separated Alexandria and Moundville from the experiment station, it was not practicable to follow the development of wilt at these locations as closely as was

done at Auburn. Consequently, from the standpoint of plant development and locational effects on wilt development, wilt counts at any given period, i. e., midseason or early fruiting stage, would not be strictly comparable for seasons within location or between locations. It will be noted, however, that seasonal differences were very much less than were locational differences. At Moundville and at Alexandria the wilt counts at midseason for 1937 were very nearly the same as for 1938. In 1939 the count was appreciably higher at both Moundville and Alexandria. About half as much wilt occurred at Auburn in 1938 as in 1936, with the infection in 1937 being intermediate.

Yields of seed cotton showed much greater seasonal variation than did wilt development. At Alexandria all varieties averaged nearly 2,000 pounds of seed cotton per acre in 1937; 1,000 pounds in 1938; and 1,250 pounds in 1939. At Moundville the yields in 1937 were almost 50% greater than in 1938 or 1939. At Auburn the seasonal variations were not very great, the lowest average yield being 745 pounds of seed cotton in 1936 and the highest being 987 pounds in 1938.

*Varieties, varieties  $\times$  locations, and varieties  $\times$  seasons.*—The classification of varieties by wilt groups was somewhat arbitrary, as may be judged by the failure to identify all of these groups by highly significant differences in the analysis of wilt or yield data by seasons and locations. It is felt that these groupings do, in the main, serve to identify varietal behavior and are useful in simplifying the discussion of varietal behavior with respect to locational and seasonal response and to potash requirement in the wilt-infested soils investigated.

As measured by the amount of wilt developed at midseason Sea Island (Seabrook) was immune from wilt at Alexandria; it was also immune at Moundville until near the end of the season when a trace of vascular discoloration was detected. Sea Island, for the one year it was included in the test, outyielded the weakly tolerant, susceptible, and very susceptible varieties at Moundville. The yield was insignificant at Alexandria.

Cook 307, Cook Wiregrass, Dixie Triumph 12, and Coker's Cleve-wilt, all assigned to the resistant group (R), usually showed the least wilt of any of the upland varieties for each season at each location, although the difference between percentage of wilt for the varieties in the resistant group and those in the highly tolerant (HT) did not reach high significance except at Moundville. At Moundville the highest yielding varieties for each season were usually in the resistant group, although in 1938 Cook 307 and Cleve-wilt 6 were exceeded by Coker's 4 in 1 and Cook 144 of the highly tolerant group. At Auburn and at Alexandria the amount of wilt, averaged for all potash treatments, in the resistant and highly tolerant varieties does not appear to have appreciably modified yield. In other words, the yielding ability of varieties in these two groups is largely independent of comparative wilt resistance, except under conditions of very severe wilt as at Moundville.

In percentage of wilt at midseason the difference between the weakly tolerant group and the average for all varieties in the highly

tolerant and resistant groups was highly significant at all three locations with minor exceptions for certain seasons. The varieties in the weakly tolerant group out-yielded the other varieties at Alexandria on the basis of the 3-year average, although within any one season certain of the resistant or highly tolerant varieties equalled or exceeded the weakly tolerant varieties.

The weakly tolerant varieties were distinctly inferior in wilt resistance and in yield to the varieties in the resistant or highly tolerant groups at Moundville. This was also true at Auburn, except for the higher rates of potash application. (See discussion under  $V \times K$  interaction.)

The susceptible varieties, Coker's 100 and Farm Relief, were clearly intermediate between the highly susceptible Half & Half and the tolerant-resistant varieties in percentage of wilt and in yield of seed cotton at Auburn and at Alexandria, although the yield of Coker's 100 exceeded certain of the tolerant-resistant varieties each year at Alexandria, and Farm Relief slightly exceeded Missdel at Auburn for two years. Half & Half averaged about twice as high in incidence of wilt as Farm Relief or Coker's 100 at Auburn and Alexandria, and was correspondingly inferior in yield. At Moundville, however, both the susceptible and highly susceptible varieties were almost completely destroyed by wilt before the end of the season and consequently gave no highly significant differences for percentage of wilt or yield.

This contrast in the behavior of the susceptible and the highly susceptible varieties at Alexandria and at Moundville is exceedingly interesting and tends to give some credence to the concept of biologic specialization of the pathogene with respect to differential pathogenicity for varieties. Based on the effect of wilt on yield, it might be concluded that Alexandria has the Half & Half type of wilt that does not affect other varieties, whereas Moundville has a form which seriously damages all upland varieties for which a 3-year average was obtained, with the exception of Cook 307, Dixie Triumph 12, and Coker's Clewewilt. When, however, the ranking of varieties with respect to percentage of wilt at all three locations is considered, it appears that the order of ranking is relatively constant regardless of season or location.

*Potash treatments,  $K \times$  location, and  $K \times$  seasons.*—In considering potash effects the interaction of variety  $\times$  potash treatments is temporarily ignored and is discussed separately under the succeeding heading. Before discussing the results from the potash treatments it may be helpful to consider the composition of the soil for the three locations with respect to potash content.

Analysis of soil samples taken from the plots in 1937 showed that they contained the following amounts of replaceable potash in pounds per acre: Auburn, 40; Moundville, 121; and Alexandria, 387. Samples of the soil from the no-potash plots were taken again at the conclusion of the regional experiment in 1939 and were analyzed by the Georgia Experiment Station in order to make a direct comparison with soils from the other regional locations. This later analysis showed that Moundville and Alexandria contained 182 and 389 pounds, respec-

tively, of replaceable potash per acre. It is of interest to note the appearance of the plants in the no-potash treatments for the 3 year's duration of the experiment at each location. At Auburn the plants in the no-potash blocks were nearly normal until early fruiting stage when severe potash deficiency symptoms began to develop. The symptoms progressed so rapidly, as pointed out in the forepart of this paper, that the detection of wilt symptoms became almost impossible due to shedding of leaves, development of necrotic branches and tops, and finally death of many plants before normal harvest season, making records of final count for wilt symptoms of questionable value. At Alexandria the no-potash plots, even after 3 years in cotton with no potash added, could not be distinguished by the appearance of the plants from the plots which had received 4 to 8% potash.

At Moundville any difference due to potash treatments was not clearly evident, although in some cases there was a distinction, which was neither consistent nor characteristic of potash deficiency, between the 0 and 8% plots. Abnormal color of the leaves and the general appearance of the plants provoked speculation as to the possible association of some other mineral deficiency or toxicity with potash treatment being responsible for the increased severity of wilt in the tolerant and resistant varieties. At Alexandria and Moundville the final wilt counts (Table 5) were not complicated by defoliation, as they were at Auburn. At both locations differences in wilt due to potash treatments were greater at final count than at midseason. Varietal differences also increased at Alexandria, but were less evident at final count at Moundville due to the exceedingly high percentage of wilt for all varieties.

At Auburn, despite the split-plot arrangement for potash treatments, the appearance of the plants and the striking results obtained leave no doubt as to the significance of treatment effects. The inadequate error (Table 2) shows significant differences for wilt at midseason in 1937 and highly significant differences for 1938. Differences in yield reached high significance for both 1937 and 1938. Three-year averages of all the varieties included in the test showed nearly twice as much wilt for the no-potash treatment as for the 4% application. There was a slight progressive reduction in the amount of wilt at midseason for 4, 8, and 16%, respectively. For yield the 3-year average for all varieties gave the highest yield with the 4% treatment. There was very little difference between the yield at 4% and 8%. The 16% treatment gave about 100 pounds decrease, while the no-potash treatment yielded little more than half the 4% treatment.

At Moundville, although potash deficiency symptoms were not clearly detectable in the no-potash treatment, there was a progressive decrease in the percentage of wilt-affected plants at midseason for all years, the 3-year average of all varieties with 0, 4 and 8% treatments being approximately 38, 32, and 29%, respectively. A significant difference was obtained between the 0 and 8% treatments in 1937 and highly significant differences were obtained between the 0 and 4% treatments in 1938 and 1939. There was a corresponding increase in yield which reached high significance for the difference between 0 and 4% treatments in 1938 and between 0 and 8% in 1939

(1937 yield data were not analyzed). It will be noted that the 3-year average for all varieties gave a yield of 355 pounds for the no-potash treatment, whereas the 8% treatment produced 612 pounds of seed cotton.

At Alexandria the requirement for high significance for reduction in wilt as a result of potash treatment was not reached during any of the three seasons, nor did any season show a highly significant increase in yield as a result of potash supplements. The 3-year average for yields of all varieties shows a slight increase in favor of potash over no-potash fertilizer from 1,360 to 1,430 pounds of seed cotton per acre. These results are not surprising as chemical analysis of the soil showed nearly 400 pounds per acre of replaceable potash.

In view of the rather high amount of replaceable potash at Moundville and the indifferent response to, or even harmful effects from, "excessive" amounts on the low-potash-content soils at Auburn, it is surprising to find the favorable response to potash, in wilt reduction and yield increase, progressing through the 8% treatment at Moundville. The close association of wilt reduction and yield increase, judged by the inverse direction of curves in Figs. 1 and 2, suggests that the beneficial effects of potash at Moundville, where wilt was sufficiently severe even in the resistant varieties to affect yield markedly, may be attributed to the potash delaying the rapid development of the disease and prolonging the life of the plant until fruiting had progressed, rather than to the needs of this soil for potash supplements for producing normally fruitful cotton plants.

*Variety  $\times$  potash and  $V \times K$  location interactions.*—In studying the data for the response of varieties to potash treatments certain rather definite trends were indicated. It was found to be rather difficult to follow the variations of 12 varieties each year at three locations; therefore, the varieties were grouped and these data are presented graphically by groups in Figs. 1 and 2 for wilt reaction at mid-season and for yield of seed cotton, respectively. The tests of significance for  $V \times K$  interaction are presented in Table 2 and are also included in the footnote references for Tables 3 and 4.

At Auburn very little wilt developed either in the three resistant or the four highly tolerant varieties. These groups did not differ from each other according to the statistical analysis made for 1937 and 1938 and accordingly were thrown together in the graphs. Although potash deficiency symptoms were very severe in the no-potash block, Fig. 1 shows that within these seven varieties potash treatments had little effect in modifying the amount of wilt. In the two varieties which were classified as weakly tolerant and the variety classified as susceptible there were marked drops in both curves for percentage of wilt between the 0 and 4% treatments, but the higher treatments had little effect on either curve. For the highly susceptible Half & Half variety, however, there was a progressive decrease through the 8 and 16% treatments.

Compared to the wilt curves in Fig. 1, the yield curves in Fig. 2 show a corresponding, although inverse, response to potash increments. There was a good response to the 4% treatment by the resistant and highly tolerant groups and a much sharper response by the

weakly tolerant group. Under the conditions of this experiment, larger amounts of potash were not beneficial for the nine tolerant or resistant varieties. For the susceptible and highly susceptible varieties greater amounts of potash appear to be required for maximum yields. The yields of the weakly tolerant group became approximately equal to those of the more resistant varieties at the 4% level of potash, at the 8% level all groups except the highly susceptible were practically indistinguishable, and at the 16% level the yields of all groups were relatively close together.

At Alexandria potash increments had comparatively little effect on the groups of varieties in modifying the amount of wilt or yield of seed cotton. There was a slight indication that increasing amounts of potash were beneficial in reducing wilt in the highly susceptible varieties and that 8% was harmful to the other groups. For yield there is, as was shown for Auburn, a slight indication that 8% was superior to 4% for the susceptible and highly susceptible varieties. The 4% treatment was slightly beneficial to all except the highly susceptible group. The slight changes in the curves and the failure to obtain a significant value for  $V \times K$  interaction, except for final wilt count in 1937, suggest that for the Alexandria experiment conclusions as to differential response of varieties to potash treatment should be made with caution. It is interesting to note that, although there was an average of nearly 40% of wilt at midseason in the susceptible Coker's 100, the yield nearly equalled that of the resistant and highly tolerant groups, particularly at the 8% potash level, and also that the highest average yield was made by the weakly tolerant group.

At Moundville the similarity of direction for the infection curves at midseason indicated little likelihood of significant differential response of varieties to potash treatment. In the analysis of variance for percentage of wilt at midseason and at final count for 3 years, the mean square for  $V \times K$  interaction was usually not much greater than that for error. Increasing amounts of potash were beneficial in reducing wilt and gave an almost straight line response for rates of  $K$  in all groups except the weakly tolerant in which the 4% appeared to be more beneficial than the 8% treatment. For yields, however, groups of varieties behaved differently with respect to each other and also gave a response to potash increments that was at variance with the corresponding response at Auburn. Under the severe wilt conditions at Moundville the groups which included the susceptible and highly susceptible varieties were least responsive to potash and those groups containing the resistant and highly tolerant varieties showed most response—a complete reversal of response to that of these groups at Auburn.

The directions of the yield curves for the weakly tolerant group were somewhat similar at Moundville and at Auburn. It is suggested that the differential behavior of varieties to potash fertilizers with respect to effects on yield at Auburn and Moundville may be associated with the comparative severity of the disease; that under the mild conditions at Auburn potash supplements serve to prevent infection or increase the longevity of infected plants in the susceptible



or highly susceptible varieties so that a fair crop results; whereas at Moundville the wilt is so severe that similar results are manifest in only the resistant and highly tolerant varieties and that no amount of potash "stimulation" is able to carry a high percentage of the plants in susceptible and highly susceptible varieties through the fruiting stage.

*General.*—Several important practical conclusions might be drawn from these experiments. It is evident that with potash-deficient soils, such as the one at Auburn where wilt is not very severe on the resistant varieties, moderate applications of potash are more profitable than applications of 8% or more unless susceptible varieties are grown. Where wilt is severe on the resistant varieties and the soils are well supplied with potash, as at Moundville, heavy applications of potash may be required for reducing wilt and only the most highly resistant varieties should be planted. Where wilt is light except on the highly susceptible varieties, as at Alexandria, potash may be of little importance and high-yielding tolerant varieties may be superior to resistant cottons.

#### RESULTS OF COTTON VARIETY TESTS IN TEN DIFFERENT LOCALITIES IN ALABAMA ON DIFFERENT TYPES OF WILT-INFESTED SOILS

For the purpose of obtaining field evidence as to the possible existence of physiological races of the wilt organism in Alabama and to determine the relative wilt resistance of varieties under widely varying conditions, 10 tests were conducted on wilt-infested soils in different localities of the state in 1936, using 21 varieties and strains of cotton which are commonly grown in Alabama and claimed to be wilt resistant. One highly susceptible variety was included as a check. The tests at four of these places, Pollard, Uriah, Eutaw, and Benton, were continued in 1937 and 1938, using 20 of the original varieties.

In 1936 different potash rates were superimposed on the test at Auburn. The potash-variety test was continued at Auburn in 1937 and 1938 but with varieties reduced to 11, one of which (Farm Relief) had not been included in 1936. Thus, at Auburn, results are available for 1 year's performance of all 22 varieties and for 3 years' performance for 10 of them. The regional-potash-variety studies were located on the Moundville and Alexandria plots in 1937 and were continued through 1939. These studies included 7 of the original 22 varieties grown in 1936 so that a 4-year history of performance is available for these 7 varieties at Alexandria and at Moundville. Eight additional varieties were included (for 1 to 3 years) in the Alexandria and Moundville studies where some information is thus provided on the performance of 31 varieties or strains.

In 1936 the cooperators furnished and applied the fertilizers for the tests at the rates and by the methods used for their own cotton crops. At Auburn the cotton was fertilized and planted as previously described for the potash-variety studies there. At the four locations where the tests were continued uniform fertilizer applications were

made in 1937 and 1938 using approximately 600 pounds of 6-8-4 per acre. At all locations except Auburn the cotton varieties, systematically arranged, were planted in one-row plots on two series in reverse order, with a susceptible variety planted in every fourth row as a check on wilt infestation. For 1937 and 1938 the checks were planted on every fifth instead of every fourth row as in 1936.

Yields were obtained on the potash-variety studies at Auburn, and on the regional potash-variety studies at Moundville and Alexandria for all three years. Yield data were not obtained from the other outlying tests. Wilt data were taken as previously described. Summaries of the wilt data taken at final count (from late August to mid-September) for the 10 tests and for the potash-variety studies at Auburn, Alexandria and Moundville are presented in Table 5.

At the locations where the studies were continued for more than 1 year for certain of the varieties the amount of wilt varied considerably with the different years. Consequently, in order to make approximate varietal comparisons in Table 5, an attempt was made to adjust the means for those varieties which were grown for fewer years at any one location to eliminate seasonal effects. The wilt was very light in 1936, particularly so on the more resistant varieties, so that varieties which were grown only in 1936 were usually adjusted upward to correspond to the average of the wilt group to which the variety belonged. Since there were only two replicates of any one variety at any location in 1936, except at Auburn, the resulting large experimental error is further magnified by the seasonal weighting and has resulted in means which are not altogether satisfactory. Despite such objections, it is felt that the table serves a useful purpose in presenting in a single place all of the data available on varietal-local performance as judged by final wilt count.

It is evident from Table 5, particularly for those varieties for which the means were not adjusted and on which in consequence the greatest reliability can be placed, that varietal ranking with respect to resistance was surprisingly constant at the several locations regardless of severity of the disease, and that the varieties in the various wilt groups fluctuated within rather narrow limits around the mean of the corresponding group by location. It may be noted that where there occurs some overlapping of varietal means with those assigned to another wilt group, such instances are generally confined to varieties which were grown for one year only at that particular location.

This uniformity of varietal ranking at widely separated locations and under varying conditions of wilt severity is interpreted as evidence that there is little reason to suspect, in Alabama, existence of strains of the pathogene which differ greatly with respect to their ability for differential pathogenicity for varieties. Consequently, if by physiological strains is meant a condition similar to that described for the rust and certain other fungi whereby varieties which are resistant at one location are susceptible at another, then no field evidence of such physiological specialization was found in these studies. It is true that the relation between different wilt groups at one location varies greatly from the corresponding relation at other locations. This may be interpreted as indicating that the wilt organism

TABLE 5.—Incidence of wilt at final count, late August-early September, in several (22 to 31) varieties and strains of cotton at 10 locations on wilt-infested soils of Alabama during four seasons, 1936 to 1939, inclusive.

Variety	Wilt group*	% wilt affected plants at final count for										
		4 years at†		3 years (1936-1938) at‡				1 year (1936 only) at*		All years and locations§		
		Moundville	Alexandria	Auburn	Uriah	Pollard	Eutaw	Benton	Slocomb		Vernon	Greenville
Means for Variety by Location												
R	Cook 307 (B. Rhyné).....	18.9	3.2	7.3	3.3	1.6	2.2	1.8	1.6	0.3	0.0	4.0
R	Cook 307 (C. Rhyné).....	16.6(1)	2.4(1)	10.3(1)	2.9	3.0	4.0	2.0	0.3	0.0	0.0	4.2
R	Cook Wiregrass.....	23.2(1)	7.8(1)	10.8	4.8	5.1	2.8	1.5	2.3	3.6	0.0	6.2
R	Dixie Triumph 12.....	31.7(3)	2.2(3)									6.7
R	Clewilt (Coker's)**.....	27.0	5.1	14.3	5.1	4.8	2.7	7.6	2.6	0.4	0.0	7.0
R	Sikes WR.....	23.7(1)	0.0(1)	16.7(1)	9.1	8.8	7.9	3.9	2.6	0.0	0.4	7.3
R	Cook 27-54.....	31.2(1)	2.4(1)	22.1(1)	5.6	6.3	4.7	5.3	2.2	0.9	1.1	8.2
R	Cook 307-92.....	44.3(1)	0.0(1)	18.4(1)	5.5(1)	3.7(1)	7.3(1)	5.8(1)	5.5	0.3	1.1	9.2
HT	Clewilt (Rhyné).....	31.2(1)	1.5(1)	6.5(1)	14.6	7.4	6.9	7.8	1.4	3.3	0.0	8.1
HT	Dixie Triumph (Maret).....	47.7(1)	0.0(1)	7.8(1)	6.8	7.4	4.2	3.0	3.2	1.4	0.0	8.2
HT	Cook 144-68.....	37.5	5.2	15.3	11.1	6.7	3.8	3.3	0.2	0.4	0.0	8.4
HT	Coker's 4 in 1.....	33.6(1)	4.2(1)									9.5
HT	Dixie 14-5.....	44.2	7.6	18.3	6.5	6.9	5.9	2.7	1.9	2.6	0.0	9.7
HT	Cook 1006.....	32.6(1)	1.5(1)	20.3	10.1	9.0	8.5	5.7	3.4	7.5	1.1	10.0
HT	Cook 144-10.....	33.6(1)	4.6(1)	26.2(1)	12.5	12.6	4.8	3.7	5.7	3.1	0.7	10.8
HT	Sikes WR Staple.....	50.3(1)	4.3(1)									11.6
HT	Dixie Triumph 85.....	46.3	4.6	14.0	16.5	8.9	10.5	8.3	2.3	2.8	2.9	11.7
HT	Toole (Perry's).....	43.2(2)	5.3(2)									12.1
HT	Cook 144-133.....	63.1(1)	4.6(1)	25.8(1)	14.0	10.6	5.6	4.6	0.3	0.9	0.0	13.0

Cook 1138.....	WT	43.8(1)	6.2(1)	9.0(1)	14.6(1)	9.6(1)	10.5(1)	0.0(1)	11.8	4.3	1.2	11.1
Missdel 1 WR.....	WT	38.9(1)	17.1(1)	19.3	29.2	17.9	11.4	10.1	7.0	6.5	1.0	15.8
Rowden 2088.....	WT	59.9(2)	13.1(1)	—	—	—	—	—	—	—	—	16.9
Rowden 12.....	WT	58.4(1)	14.4(1)	—	—	—	—	—	—	—	—	17.4
Deltapine 12.....	WT	88.8(1)	19.2(1)	25.8(1)	9.6	2.6	6.5	5.4	11.7	20.4	10.3	18.0
Cleveland (Warm).....	WT	88.8(1)	16.0(1)	28.3(1)	18.4	12.4	11.7	5.0	15.2	12.3	9.0	19.3
Dixie Triumph (Warm).....	WT	65.0(1)	27.0	28.0	34.4	20.4	12.4	17.2	14.1	5.9	1.0	19.6
Miller 610.....	WT	61.2	12.5	—	—	—	—	—	—	—	—	20.0
Deltapine A.....	WT	59.7(1)	21.1(1)	—	—	—	—	—	—	—	—	22.1
Deltapine 3.....	WT	58.6(1)	21.2(1)	—	—	—	—	—	—	—	—	42.0
Coker's 100.....	WT	70.0(3)	46.0(3)	33.1(1)	43.9	23.2	14.7	15.5	6.0	4.5	0.5	42.6
Farm Relief.....	S	—	—	53.5(2)	—	—	—	—	—	—	—	—
Check††.....	HS	83.0	63.7	69.8	81.1	54.9	45.3	49.3	71.5	51.0	21.2	59.1

Means for Locations Based on the 22 Varieties Common to all Locations

## Locational Averages for Wilt Groups

R	27.1	2.9	14.3	6.4	5.3	4.7	4.3	2.4	1.1	0.3	6.9
HT	42.1	3.9	16.8	11.8	9.1	6.5	4.7	2.3	2.9	0.8	10.1
WT	56.1	15.9	23.8	25.0	14.2	11.2	8.9	9.3	9.0	3.8	17.7
S	70.0	46.0	53.5	—	—	—	—	—	—	—	42.1
HS	83.0	63.7	69.8	81.1	54.9	45.3	49.3	71.5	51.0	21.2	59.1

\*"Wilt Group" represents the classification for wilt resistance which was assigned on the basis of performance at the 10 locations in Alabama in tests which were conducted from 1936 to 1939, inclusive. The symbols R, HT, WT, S, and HS signify resistant, highly tolerant, weakly tolerant, susceptible, and highly susceptible, respectively.

†Actual means are used for the varieties which were grown for 4 years at Moundville and Alexandria. For varieties which were grown 1, 2, or 3 years only, figures in parentheses are given to indicate the number of seasons varieties were included in the tests. For varieties so designated means are weighted for seasons to permit approximate comparison of performance. Actual means are given for the varieties which were grown for 3 years. The two varieties grown 1 year only are indicated by the figures in parentheses and the means are weighted for seasonal effects.

‡The average percentage of wilt for all years and locations represent the mean value for varietal performance (freed of seasonal effects) at all locations. Values for varieties which were grown at Moundville, Alexandria, and Auburn only are weighted for locational effect.

§The Cheyenne strain 3 was used in 1936; strain 6 in 1937 and 1938; strain 7 in 1939. These strains were so similar with respect to resistance that they are considered a single variety. The average percentage of wilt for all years and locations except Auburn. At Auburn in 1936 and at all locations thereafter Half & Half was used. Both varieties were highly susceptible and no attempt has been made to distinguish between their performance.

in one locality is decidedly more virulent than it is in another. Accepting this as one type of biologic specialization of the pathogene, the field evidence does suggest that there may be races of the pathogene which differ from each other in virulence or their capacity to parasitize cotton varieties in general, especially those belonging to the tolerant and resistant groups. If, however, the basis for interpreting the field data is the hypothesis that the ratios of disease in various wilt groups indicate races of the pathogene which are more or less "virulent", then certain possible contributing factors should be considered. At Moundville, for example, there was a heavy infestation of root knot nematodes and the appearance of the plants indicated an undiagnosed physiologic disturbance. Under such environmental conditions it appears doubtful that the severity of the disease in the resistant-tolerant varieties should be ascribed to a virulent race of the pathogene. This is further emphasized by the seasonal variations in ratios for a given location (as used for obtaining weighted means in Table 5). At Moundville the ratios for the wilt groups, R, HT, WT, and HS, respectively, were in 1936, 5, 13, 26, 91; in 1937, 44, 59, 73, 88; in 1938, 31, 53, 64, 72; and in 1939, 23, 45, 68, 81.

In considering locational effects and the possible influence of potash deficiency which may not have been corrected by the fertilizer application indicated, it may be pointed out that chemical analysis of soil samples taken in 1937 showed the following amounts of replaceable  $K_2O$  per acre: Auburn, 40 pounds; Slocomb, 31 pounds; Pollard, 51 pounds; Uriah, 53 pounds; Greenville, 44 pounds; Benton, 87 pounds; Moundville, 121 pounds; Eutaw, 69 pounds; and Alexandria, 387 pounds.

#### SUMMARY

From the several field experiments which were conducted on wilt-infested plots for from 1 to 4 years at diverse locations in Alabama several conclusions of practical interest to agronomists, cotton breeders, and other investigators interested in the *Fusarium* wilt disease of cotton appear to be warranted.

These experiments as a group indicated that the development of wilt varies greatly between locations in the state within years, by seasons within locations, and between varieties within locations by seasons.

At locations where experiments were conducted for 3 years or more the average amount of wilt developed in the highly susceptible group showed a range of 45 to 83% of wilt-affected plants at final count. Much fluctuation occurred within the weakly tolerant group with average percentages ranging from 9 to 56. At most of the locations the resistant and highly tolerant groups were distinguishable only with difficulty, while at other locations, Moundville, Uriah, and Pollard, the resistant group was clearly superior to the highly tolerant group.

The increased severity of infection in the tolerant-resistant groups at Uriah, Pollard, and Auburn may be attributed to a deficiency of potash (50 pounds or less of replaceable  $K_2O$  per acre), but at Moundville potash deficiency symptoms were not evident (chemical analysis

showed more than 100 pounds of replaceable  $K_2O$  per acre). Root knot nematodes, however, were severe at Moundville and may have been a contributing factor to the severity of the disease.

The relatively constant ranking of varieties or strains of cotton with respect to percentage of wilt at various locations in Alabama, regardless of general wilt severity, is interpreted as field evidence against the existence of physiological races of the pathogene which differ greatly in their ability to parasitize specific varieties. The differential response of varieties to potash, the influence of seasons and other environmental factors are indicated as of greater practical concern than are possible races of the pathogene that would complicate the breeders program.

These experiments indicate that breeding plots which might be located where conditions are similar to those prevailing at Alexandria would be worthless except for eliminating highly susceptible strains.

The potash-variety-wilt studies at Auburn, Moundville, and Alexandria showed that varieties differ with respect to potash needs for normal development or for withstanding wilt attacks. This differential response of varieties to potash treatments appears to be associated with the grouping of varieties with respect to resistance. At Auburn and Alexandria, where the wilt was not severe except on the susceptible and highly susceptible varieties, these varieties continued to respond favorably to increasingly heavy applications of potash. Where wilt was exceptionally severe, as at Moundville, heavy applications of potash did not enable such varieties to overcome wilt. At Moundville heavy applications of potash were of greatest value to the resistant varieties. On the other hand, 3 years' results at Auburn showed that resistant varieties require only a moderate amount of potash for maximum yields and that greater amounts are harmful.

There was a rather close, but inverse, relationship between yield of seed cotton and the percentage of wilt-affected plants at the mid-season count.

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## COLOR INHERITANCE IN BARLEY<sup>1</sup>

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IN connection with investigations of awn inheritance and mildew resistance in barley, a number of crosses involving color differences in pericarp, aleurone, and endosperm were made. In examining this material, it was discovered that blue aleurone was produced by complementary factors which had not previously been reported. It therefore appeared advisable to make a further examination of color inheritance in barley.

Harlan (3)<sup>3</sup> reported that colors in barley were caused by two pigments; one, anthocyanin, is violet in its acid condition and blue in its alkaline condition; the other, a melanin-like compound, is black. These pigments may occur in the hulls, the pericarp, the aleurone layer, and occasionally in the endosperm. White denotes the absence of either pigment. Anthocyanin in the pericarp alone results in a violet color, while in the aleurone alone it results in a blue color. The acid condition of the anthocyanin in the pericarp superimposed upon the alkaline condition in the aleurone gives the effect of a purple. In the absence of the pigment in both the pericarp and the aleurone layer, the kernel is white.

Daane (2) reported purple vs. white pericarp (Pp) as due to a single factor. He found the factor Pp linked to normal vs. virescent seedlings (Aa) with 17.9% crossing over. He also found (Aa) was linked with non-six-rowed vs. six-rowed (Vv), which thus placed the factor (Pp) in linkage group I.

Miyake and Imai (5), in studying purple hull, reported a two-factor difference between deep purple and white hull. They report a 9 deep purple:6 light purple:1 white segregation in one cross. In a similar cross a 15 purple:1 white were reported where deep purple and light purple could not be classified separately. In a third cross, light purple and white could not be separated and resulted in a 9 deep purple:7 light purple segregation. One of the purple factors (P<sub>1</sub>P<sub>1</sub>) was shown to be linked with two-rowed vs. six-rowed, a factor for long awn, and a factor for dwarf early plant type.

A number of authors have reported blue vs. white aleurone as due to a single factor. Buckley (1) reported this factor, Blbl, in group IV linked with hooded vs. awned (Kk) with a crossover value of 40.6%. Robertson, *et al.* (6), with considerably more data, placed it in the same group with a crossover value of 22.58% with the factor for hooded vs. awned (Kk).

Buckley (1) also reports the inheritance of white vs. orange lemma (Oo) as being due to a single factor which is linked with long vs. short haired rachilla (Ss) with a crossover value of 39.1%. This places Oo in linkage group V.

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 436.



## MATERIALS

Blackhulless and an unnamed variety, C.I. No. 5628, are the purple parents used in this study. Both these varieties have a purple pericarp underlain by a blue aleurone layer. The four varieties Awnless, Kwan, Algerian, and Bolsheviki have blue aleurone layers and no pigment in the pericarp. Nepal, Goldfoil, and Hanna, have no pigment in either the pericarp or aleurone layer and are thus a white or cream color. An unnamed variety, C.I. No. 5649, has no pigment in the pericarp or aleurone layer but does have an orange pigment in the lemma and palea, and this pigment also shows in the rachis. Atlas, a variety with a white lemma, was also used in the study of the inheritance of orange lemma.

These 11 varieties, together with their contrasting characters used in the genetic studies, are shown in Table 1.

TABLE 1.—*Parent varieties and characters studied in each.*

Variety	C. I. No.	Kernel Color	Hulled or naked	Hooded or awned	Fertility of lateral florets	Lemma color	Character of awn	Rachilla hair length
Blackhulless....	666	Purple	Naked	Awned	6-row	White*	Rough	—
	5628	Purple	Naked	Awned	6-row	White*	Rough	—
Kwan....	1016	Blue	Hulled	—	—	—	—	—
Algerian....	1179	Blue	Hulled	—	—	—	—	—
Awnless....	5631	Blue	Hulled	Awnless	6-row	White	Rough†	—
Bolsheviki	—	Blue	—	Awned	6-row	White	Rough	—
	5649	White	Hulled	Awned	2-row	Orange	Smooth	Long
Nepal....	595	White	Naked	Hooded	6-row	White	Rough‡	—
Goldfoil....	928	White	Hulled	—	—	—	—	—
Hanna....	906	White	Hulled	—	—	—	—	—
Atlas....	4118	—	—	Awned	6-row	White	Rough	Short

\*Traces of purple.

†Carries factor for rough, though awn not present.

‡Carries factor for rough awn, though masked by hood.

## EXPERIMENTAL RESULTS

## PURPLE VS. BLUE

In three crosses with the purple parent, Blackhulless, and the blue parents, Algerian, Kwan, and Awnless, there was a single factor difference between purple and blue (Pp). The cross between the unnamed purple variety, C.I. No. 5628, and Bolsheviki, which has a blue aleurone, also shows a single factor difference, as is shown in Table 2.

Classification of plants for purple (Pp) is very difficult in some of the hulled plants. In most cases the purple shows in the lemma, but in some of the plants which presumably are heterozygous no purple shows in the lemma. These purples are much less intense and often show purple only near the kernel tip. When this mere trace of purple is underlain by a dark blue aleurone and overlain by a heavy lemma it is most difficult to classify. Thus, the low probability in the Kwan and Awnless crosses may be accounted for by the difficulty of classifying hulled purple plants. If only the naked segregates are considered,

TABLE 2.—*Segregation for purple vs. blue in four crosses involving four blue varieties and two purple varieties grown at Davis, Calif., in 1940 and 1941.*

Cross	Purple	Blue	$\chi^2$	P
All Plants				
Algerian $\times$ Blackhullless	348	96	2.703	.1
Kwan $\times$ Blackhullless	415	166	4.053	.02-.05
Awnless $\times$ Blackhullless	595	234	4.694	.02-.05
Bolsheviki $\times$ C. I. No. 5628	319	119	1.099	.2 -.3
Plants with Naked Caryopsis				
Algerian $\times$ Blackhullless	88	25	0.423	.5 -.7
Kwan $\times$ Blackhullless	102	44	1.774	.1 -.2
Awnless $\times$ Blackhullless	149	50	0.000	.9 -.95
Bolsheviki $\times$ C. I. No. 5628	77	30	0.527	.3 -.5

as shown in Table 2, there is a very good fit to the expected ratio on the basis of a single factor difference.

## PURPLE VS. WHITE

Four crosses between purple and white parents segregate 12 purple: 3 blue:1 white. The data on these four crosses are given in Table 3.

TABLE 3.—*Segregations in four crosses involving purple and white varieties grown at Davis, Calif., in 1940 and 1941.*

Cross	Purple	Blue	White	$\chi^2$	P
Goldfoil $\times$ Blackhullless	401	90	42	3.457	.1-.2
Hanna $\times$ Blackhullless	480	104	43	2.284	.3-.5
C. I. No. 5649 $\times$ Blackhullless	434	113	26	3.018	.2-.3
C. I. No. 5649 $\times$ C. I. No. 5628	266	64	24	0.250	.8-.9

The data presented in Table 3 show a two-factor difference in color segregation. The purple varieties also carry a blue aleurone, while the white varieties carry the recessive for both purple and blue. The ratio obtained is 12 purple:3 blue:1 white. This indicates that the factors for purple pericarp and blue aleurone are independent. One would expect a ratio of 9 purple:3 violet:3 blue:1 white, if Harlan's (3) explanation of color held, that is, that purple is the result of violet pericarp underlain by a blue aleurone layer. In these crosses no attempt was made to separate the purple plants with a blue aleurone from the plants carrying the purple factor and a colorless aleurone. Thus, these two classes were combined in this study.

## LINKAGE RELATIONSHIPS OF PURPLE FACTOR (Pp)

In crosses of the two purple parents, Blackhullless and C.I. No. 5628, with the white two-rowed variety, C.I. No. 5649, the segregation for purple vs. non-purple (Pp) and non-six-rowed vs. six-rowed (Vv) varied markedly from the expected 9:3:3:1 ratio. Table 4 shows the segregation observed, the expected frequency on a 9:3:3:1 basis, and the frequency calculated from the crossover percentages

of the two crosses. Immer's (4) tables were used to calculate the linkage intensities.

TABLE 4.—Interaction between purple vs. non-purple (*Pp*) and non-six-rowed vs. six-rowed (*Vv*) in two crosses grown at Davis, Calif., in 1940 and 1941.

Phenotype	Observed	Expected on 9:3:3:1 basis	Expected with linkage
Blackhullless × C. I. No. 5649			
Purple, non-six-rowed.....	293	322.4	Expected with 12.09% C.O. 288.7
Purple, six-rowed.....	141	107.4	141.1
Non-purple, non-six-rowed.....	137	107.4	141.1
Non-purple, six-rowed.....	2	35.8	2.1
	$\chi^2$	53.262	.7645
	P	Very low	.5-.7
C. I. No. 5628 × C. I. No. 5649			
Purple, non-six-rowed.....	177	199.1	Expected with 14.80% C.O. 178.9
Purple, six-rowed.....	86	66.4	86.6
Non-purple, non-six-rowed.....	89	66.4	86.6
Non-purple, six-rowed.....	2	22.1	1.9
	$\chi^2$	34.212	.098
	P	Very low	.95-.98
Combined			
Purple, non-six-rowed.....	470	521.5	Expected with 13.19% C.O. 467.6
Purple, six-rowed.....	227	173.8	227.7
Non-purple, non-six-rowed.....	226	173.8	227.7
Non-purple, six-rowed.....	4	57.9	4.0
	$\chi^2$	87.225	.027
	P	Very low	.98-.99

The probability for independent inheritance in both crosses is extremely low. When linkage values together with their probable errors are calculated from Immer's tables, the crossover percentage in the first cross is  $12.09 \pm 2.76$  and in the second cross  $14.80 \pm 3.48$ . In a test for homogeneity of the two crosses a high probability is obtained. Therefore, the data from these two crosses can be combined and a single linkage value determined. This linkage value and its probable error is  $13.19 \pm 2.17\%$ . This linkage is in agreement with Daane (2) who found the factors *Pp* and *Vv* linked to a gene (*Aa*) for normal vs. virescent seedlings. Daane, however, made no report of a direct study of linkage between the factors *Pp* and *Vv*.

In these two crosses Pp was found to be independent of hulled vs. naked (Nn), group III; rough vs. smooth awn (Rr), group V; and white vs. orange lemma (Oo), unplaced.

#### BLUE VS. WHITE ALEURONE

Three blue varieties, Kwan, Algerian, and Awnless, when crossed with Nepal segregates 3 blue:1 white, as shown in Table 5.

TABLE 5.—*F<sub>2</sub> segregation for blue vs. white in three crosses grown at Davis, Calif., in 1940.*

Cross	Blue	White	$\chi^2$	P
Kwan × Nepal	519	171	0.008	.9-.95
Algerian × Nepal	284	105	.879	.3-.5
Awnless × Nepal	675	207	1.103	.2-.3

The data presented in Table 5 show a single factor difference for blue vs. white aleurone, which is in agreement with Buckley (1) and Robertson, *et al.* (6).

#### WHITE VS. WHITE ALEURONE

A cross between two white varieties, Goldfoil and Nepal, segregated 9 blue:7 white. In a population of 795 plants there were 426 blue and 369 white segregates. This gives a  $\chi^2$  of 2.254 and a probability between .1 and .2 on the basis of a 9:7 ratio. This cross shows that there are two factors for blue aleurone which are complementary to each other. Further, in a cross between Hanna and Goldfoil, the  $F_1$  is white, showing that the single factor for blue in Hanna and Goldfoil must be the same.

Thus the 3:1 ratio between blue and white aleurone reported by Buckley (1) and Robertson, *et al.* (6), and also reported above, is the result of crossing a blue aleurone variety which contains both blue factors, with a white variety carrying one blue factor. Therefore, segregation takes place for only one factor.

#### LINKAGE RELATIONS OF BLUE FACTORS

As previously mentioned, Robertson, *et al.* have shown a factor for blue vs. white aleurone (B1b1) is linked with the factor for hooded vs. awned (Kk). In the crosses Algerian × Nepal and Kwan × Nepal, it is possible to test the interaction of the factor for blue carried by Algerian and Kwan and not carried by Nepal, and the factors for hooded vs. awned (Kk) and for hulled vs. naked (Nn). In the cross Awnless × Nepal, it is not possible to test for linkage between blue and hooded, since in this cross the hooded segregates must carry two awn factors,<sup>4</sup> but this cross is useful in testing the interrelation of the blue and hulled factors. In the cross Goldfoil × Nepal, where complementary factors for blue were shown to be present, it is possible to test the segregation of blue with both Kk and Nn.

<sup>4</sup>Unpublished data of J. L. Myler on awn inheritance in barley.

The data for the interaction of blue vs. white and hooded vs. awned in the crosses Algerian  $\times$  Nepal, Kwan  $\times$  Nepal, and Goldfoil  $\times$  Nepal are shown in Table 6. In the cross Algerian  $\times$  Nepal the probability falls below the 5% point, but this is largely due to the poor ratio between hooded and awned in which case the probability was less than 2%. These data show the factor for blue vs. white carried by Algerian and Kwan is independent of the factor for hooded vs. awned (Kk). Therefore, this is not the blue factor reported by Robertson, *et al.*, (6) and Buckley (1). It will be shown later that the variety Awnless also differs from Nepal by this same factor for blue and, therefore, the three varieties Algerian, Kwan, and Awnless all differ from Nepal by the same factor for blue.

TABLE 6.—Observed ratios in *F*, for inheritance of blue vs. white and hooded vs. awned in crosses grown at Davis, Calif., in 1940 and 1941.

Phenotype	Cross					
	Algerian $\times$ Nepal		Kwan $\times$ Nepal		Goldfoil $\times$ Nepal	
	Obs.	Exp. 9:3:3:1	Obs.	Exp. 9:3:3:1	Obs.	Exp. 27:9:21:7
Blue hooded.....	191	218.9	399	388.1	361	335.4
Blue awned.....	93	72.9	120	129.4	65	111.8
White hooded.....	80	72.9	129	129.4	221	260.9
White awned.....	25	24.3	42	43.1	148	86.9
$\chi^2$ .....	9.772		0.908		70.641	
P.....	.02-.05		.8-.9		Very low	

Since the observed ratios in the Goldfoil  $\times$  Nepal cross vary markedly from expected, Immer's tables were used to calculate a linkage value for the factor carried by the Nepal parent. This linkage value and its probable error is  $24.72 \pm 1.73\%$ . When the expected classes are calculated on the basis of the above crossover percentage, a  $\chi^2$  of 2.846 and a probability between .2 and .3 are obtained. This agrees closely with Robertson's estimate of  $22.58 \pm 0.82\%$  crossing over for the factor Blbl with Kk. Thus, the blue factor carried by Nepal must be the same factor as reported by Robertson, *et al.* (6).

In the above-mentioned four crosses it is possible to test for linkage between blue and hulled. Very poor fits are obtained both for the 9:3:3:1 ratio expected in the first three crosses and the 27:21:9:7 ratio expected in the fourth cross, as shown by the data presented in Table 7.

From the data presented in Table 7 it is apparent that linkage is involved in all four crosses. Using Immer's tables for calculating linkage and probable error, we obtain the following values for the crosses:

	Linkage, %	P
Algerian $\times$ Nepal	$8.32 \pm 0.99$	.1-.2
Kwan $\times$ Nepal	$7.16 \pm 0.69$	.7-.8
Awnless $\times$ Nepal	$11.37 \pm 0.77$	.3-.5
Goldfoil $\times$ Nepal	$11.73 \pm 1.22$	.2-.3

TABLE 7.—Observed ratios in  $F_2$  for inheritance of blue vs. white and hulled vs. naked in crosses grown at Davis, Calif., in 1940.

Phenotype	Cross							
	Algerian × Nepal		Kwan × Nepal		Awnless × Nepal		Goldfoil × Nepal	
	Obs.	Exp. 9:3:3:1	Obs.	Exp. 9:3:3:1	Obs.	Exp. 9:3:3:1	Obs.	Exp. 27:9:21:7
Blue hulled.....	273	218.9	495	388.1	631	496.1	393	335.4
Blue naked.....	11	72.9	21	129.4	44	165.4	33	111.8
White hulled....	22	72.9	26	129.4	48	165.4	188	260.9
White naked....	83	24.3	147	43.1	159	55.1	181	86.9
$\chi^2$ .....	243.267		379.151		403.037		187.698	
P.....	Very low		Very low		Very low		Very low	

If a combined linkage value is computed from all four crosses by the method of maximum likelihood, a value of  $9.88 \pm 0.44\%$  is obtained. The test for homogeneity of these crosses gives a high probability, indicating that we are justified in grouping them to arrive at a combined linkage value.

In the cross, Blackhulless × C. I. No. 5649, it is possible to test the blue and white segregates for linkage with the factor for hulled vs. naked (Nn). In this cross there were 88 blue hulled:25 blue naked:19 white hulled:7 white naked plants, where the expected numbers were 78:26:26:9. This gives a  $\chi^2$  of 3.650 and a P value of .3-.5. In the unnamed variety, C. I. No. 5628 × C. I. No. 5649 the blue vs. white segregates are also independent of the hulled vs. naked segregates, giving 46 blue hulled:18 blue naked:18 white hulled:6 white naked, where 49.5:16.5:16.5:5.5 are the numbers expected. Thus, since the blue segregates are independent of the factor Nn, the blue factor carried by C. I. No. 5649 must be B1B1, and is thus the same blue factor as carried by Nepal.

#### WHITE VS. ORANGE LEMMA

In six crosses involving the unnamed variety C. I. No. 5649, there was a single factor difference between white vs. orange lemma (Oo) which is in agreement with the results obtained by Buckley (1). The  $F_2$  data for these crosses are shown in Table 8.

TABLE 8.— $F_2$  segregation for white vs. orange lemma in six crosses grown at Davis, Calif., 1939 to 1941.

Cross, C. I. 5649 with	White	Orange	$\chi^2$	P
Nepal.....	352	115	0.045	.8-.9
Blackhulless.....	424	149	0.336	.5-.7
C. I. 5628.....	257	97	1.088	.2-.3
Atlas.....	415	127	0.707	.3-.5
Awnless.....	289	80	2.168	.1-.2
Bolshevik.....	395	119	3.097	.05-.1

## LINKAGE RELATIONSHIPS OF THE FACTOR FOR ORANGE LEMMA (Oo)

The factor interactions between white vs. orange lemma (Oo) and characters representing linkage groups I, III, IV, and V are shown in Table 9.

TABLE 9.—*Inheritance involving the factor pair for white vs. orange lemma, F<sub>2</sub> data from crosses grown at Davis, Calif., 1939 to 1941.*

Cross, C. I. 5649 with	Characters	AB	Ab	aB	ab	$\chi^2$	P
Nepal.....	Oo and Rr	72	19	17	11	3.872	.2-.3
Blackhulless.....	Oo and Rr	262	76	91	28	1.522	.5-.7
C. I. 5628.....	Oo and Rr	189	68	75	22	1.665	.5-.7
Atlas.....	Oo and Rr	312	104	100	26	2.088	.5-.7
Awnless.....	Oo and Rr	201	67	54	15	3.891	.2-.3
Bolsheviki.....	Oo and Rr	225	70	88	31	3.414	.3-.5
Total.....	—————	1261	404	425	133	0.893	.8-.9
Atlas.....	Oo and Ss	320	95	88	39	3.071	.3-.5
Nepal.....	Oo and Vv	259	93	84	31	0.649	.8-.9
Blackhulless.....	Oo and Vv	323	101	107	42	1.457	.5-.7
C. I. 5628.....	Oo and Vv	189	68	74	23	1.458	.5-.7
Atlas.....	Oo and Vv	321	94	94	33	2.010	.5-.7
Awnless.....	Oo and Vv	225	64	64	16	4.243	.2-.3
Bolsheviki.....	Oo and Vv	224	71	82	37	5.907	.1-.2
Total.....	—————	1541	491	505	182	1.685	.5-.7
Blackhulless.....	Oo and Pp	321	103	113	36	0.479	.9-.95
C. I. 5628.....	Oo and Pp	198	59	68	29	3.024	.3-.5
Total.....	—————	519	162	181	65	1.982	.5-.7
Blackhulless.....	Oo and Bl,bl <sub>1</sub>	84	19	29	7	3.213	.3-.5
C. I. 5628.....	Oo and Bl,bl <sub>1</sub>	40	19	24	5	5.656	.1-.2
Total.....	—————	124	38	53	12	3.453	.3-.5
Nepal.....	Oo and Nn	267	85	90	25	0.870	.8-.9
Blackhulless.....	Oo and Nn	330	94	104	45	4.291	.2-.3
C. I. 5628.....	Oo and Nn	180	77	72	25	4.377	.2-.3
Total.....	—————	777	256	266	95	0.974	.8-.9
Nepal.....	Oo and Kk	261	91	87	28	0.647	.8-.9

In Table 9 the interaction of white vs. orange lemma (Oo) is shown with two characters in linkage group V, rough vs. smooth awn (Rr) and long vs. short haired rachilla (Ss). These two characters, Rr and Ss, show a crossover value of about 23% in the Atlas cross which shows they are the two characters previously reported as marker genes for group V. Buckley (1) has reported the factor Oo as being in group V with a crossover value of about 39.1% with the factor Ss. The data in Table 9 show independence between the factor Oo and both Rr and Ss. The interaction of Oo and Rr is shown in six crosses, each of which show a satisfactory probability, and when the

six crosses are combined, the probability is above .8. The interaction between Oo and Ss is independent, as shown in Table 9, which according to Buckley's results should show linkage with about 39% crossing over. If Buckley's results were correct, there should be a fairly close linkage between Oo and Rr which is not in agreement with the above results. Buckley's conclusions were based on a small population (60 plants) which may account for the difference between his results and those presented here. Thus, the evidence here presented is fairly conclusive that white vs. orange lemma (Oo) is not in group V.

The results shown in Table 9 also show the factor Oo is not in linkage group I, as it is inherited independently of both non-six row vs. six row (Vv) and purple vs. non-purple pericarp (Pp).

Two factors in group III, one for blue vs. white aleurone (B1bl1) and hulled vs. naked (Nn) are shown to be independent of the factor Oo. The factor for hooded vs. awned (Kk) representing group IV is shown to be independent of Oo by the last cross in Table 9.

### SUMMARY

It was shown that the two purple pericarp parents, Blackhullless and C. I. No. 5628, have the same factor for purple (Pp) which is linked with non-six-rowed vs. six-rowed (Vv) with 13.19% crossing over. The factor Pp is thus in group I.

Two complementary factors for blue aleurone were reported. One of these factors, previously studied by Robertson, *et al.* (6) and Buckley (1) and designated as B1bl1, is linked with the hooded vs. awned character (Kk). The value  $24.72 \pm 1.73\%$  crossing over does not differ significantly from Robertson's value of  $22.58 \pm 0.82\%$  crossing over. This factor has been placed in linkage group IV.

A second factor for blue, which will be designated B1bl1, is found to be linked with the hulled vs. naked character (Nn) which places it in linkage group III. The crossover value for this linkage is  $9.88 \pm 0.44\%$ .

The factor for white vs. orange lemma (Oo) was shown to be inherited independently of factors representing linkage groups I, III, IV, and V.

The factorial constitution of the varieties studied with regard to pericarp and aleurone color is given below:

Purple pericarp	Blackhullless	PP, B1B1, B1B1
	C. I. No. 5628	PP, B1B1, B1B1
Blue aleurone layer	Algerian	pp, B1B1, B1B1
	Kwan	pp, B1B1, B1B1
	Awnless	pp, B1B1, B1B1
	Bolshevik	pp, B1B1, B1B1
White aleurone layer	Goldfoil	pp, blbl, B1B1
	Hanna	pp, blbl, B1B1
	Nepal	pp, B1B1, blbl
	C. I. No. 5649	pp, B1B1, blbl



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POTATO QUALITY: V. RELATION OF TIME OF PLANTING,  
TIME OF HARVEST, AND FERTILIZER TREATMENT  
TO COMPOSITION AND COOKING QUALITY<sup>1</sup>

ORA SMITH AND L. B. NASH<sup>2</sup>

EVIDENCE has been reported that the time of planting is important in the production of mealy potatoes. Findlay (2)<sup>3</sup> states that early-planted, sprouted seed produced dry mealy potatoes, whereas late-planted, unsprouted seed of the same varieties produced very soggy tubers. Metzger (3) reported that the Russet Burbank variety planted between May 2 and May 25 produced tubers of better quality than those planted at later dates. Results which have been obtained in Michigan (4) indicate that early-planted seed produces better potatoes than that planted late.

It has been shown by Nash and Smith (7) that more mealy tubers are produced in light of high intensity than of lower intensity. Also, it has been stated by Cobb (1) that relatively low temperatures favor the production of mealy tubers. From a climatological standpoint, high light intensity and low temperature would not usually occur simultaneously during the growing season in New York State and thus would tend to oppose each other in the formation of high quality tubers at most times during the growing season. If the period of maturity is delayed until late September or early October, as is the case with New York grown Rurals, the length of the days is shorter, light intensity likely to be less, and the average temperature lower.

Nash (6) and Nash and Smith (7) have also pointed out that low light intensity and low temperatures may be important in the production of tubers which blacken after boiling. This being the case, one would expect that tubers which matured under conditions of high light intensity and high temperatures would blacken less than those produced under conditions of low light intensity and lower temperatures.

It has been pointed out by numerous investigators that immature tubers are lower in dry weight and less mealy than mature tubers. However, few, if any, results concerning the relation between maturity and specific gravity have been published.

Much has been published on the effect of fertilizers on quality; however, the evidence is so conflicting that little is known with much certainty concerning the effect of mineral nutrition on texture or color in potatoes. Certain investigators (5, 9) have stated that potassium is important in preventing blackening. The evidence with regard to the effect of potassium on texture is preponderantly in favor of the idea that potassium lowers specific gravity and meakness. However, it is a question as to whether or not in most of these experiments the accompanying chloride was largely responsible. Equally good evi-

<sup>1</sup>Paper No. 240, Department of Vegetable Crops, Cornell University, Ithaca, New York. Received for publication December 4, 1941.

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 451.

dence is available to show that potash does not lower quality. The effects of nitrogen and phosphorus on color and texture are likewise unsatisfactorily determined.

To determine the effects of light and temperature which prevail under field conditions during the latter stages of growth of potatoes on cooking quality, a series of fertilizer treatments were made at each of three different planting dates in order to obtain tubers at various stages of maturity when certain light and temperature conditions prevail. In addition, tubers of each treatment were harvested at three different dates during the growing season.

## MATERIALS AND METHODS

The fertilizer treatments involved applications of the following analyses: 5-10-0, 5-10-5, 5-10-10, 0-10-10, 10-10-10, 5-0-10, 5-20-10, 5-10-10 plus three side dressings of 2% nitrogen, 5-10-10 plus three side dressings of 2% potash. The rate of application in all treatments was 1,000 pounds per acre and the fertilizer was applied in the row at the time of planting. In addition, one plot received no fertilizer, thus making a total of 10 treatments. On May 15, June 1, and June 15, 1940 complete sets of these 10 treatments were planted with Smooth Rural potatoes. The plots were six rows wide and 30 feet long. On August 16, September 7, and October 5, one-third of each plot was harvested and the tubers used for specific gravity measurements, dry weight, and cooking tests.

Specific gravity was measured and samples for subsequent determinations were selected by using a series of sodium chloride solutions, as mentioned by Smith and Nash (8). The difference in specific gravity between successive solutions was 0.004.

Dry weight determinations were made in duplicate. Eight tubers from the average specific gravity of each lot were used for these determinations.

Eight tubers were selected in a similar manner for the cooking tests which were run in duplicate. Partially standardized conditions for boiling were used. With regard to the texture, a rating of 10 was given to samples which exhibited a high degree of mealiness and a rating of 1 to samples which were extremely soggy. Likewise, a rating of 10 was given to tubers which remained white for one-half hour after boiling and a rating of 1 for those which blackened very seriously. All the samples from the last harvest were subjected to boiling tests; however, from the first two harvests, samples from only four of the fertilizer treatments were boiled.

## RESULTS

### EFFECT OF TIME OF PLANTING

It may be noted from the results presented in Tables 1 and 2 that only at the first harvest (August 16) were there consistent differences in specific gravity and dry weight between tubers from the different dates of planting. Those tubers at the first harvest from the first planting were consistently highest in specific gravity and dry weight and those from the last planting consistently the lowest. No consistent difference in specific gravity or dry weight could be detected between samples at the last two harvests from plots planted at the three dates.

The time of planting had no consistent effects on the occurrence of blackening (Table 3).

At the first harvest, in general, the more mature tubers were of a more mealy texture than those planted later and less mature (Table 4). Likewise, at the second harvest they ranked in the same order; however, the difference between the samples from the three plantings was much less than that of the first harvest. At the last harvest there were no great differences in texture between the samples from the three plantings, although there was a tendency for the early-planted tubers to be more mealy than those planted later.

#### EFFECT OF DATE OF HARVEST

The results presented in Table 1 indicate some differences in specific gravity of tubers when harvested at different times. When tubers from the May 15 planting are compared at the three harvest dates there is a tendency for those of the first harvest to be slightly lower than those of the second and third harvests. Because of immaturity of tubers of the June 1 and June 15 plantings at the first harvest, their specific gravity is consistently lower than those of the same dates of planting at the second and third harvests. Because of this low specific gravity of the tubers of the second and third planting at the first harvest, the average specific gravity for all treatments of the first harvest (1.079) is lower than that of the second (1.094) and third harvests (1.092). Young tubers are always lower in specific gravity, dry weight, and starch than older tubers of the same variety grown under the same environmental conditions.

The data in Table 2 indicate similar differences in dry weight of tubers of the three harvests. Tubers of the June 1 and June 15 plantings at the first harvest are consistently lower in percentage dry weight than those of the same dates of planting at the second and third harvests. The average dry weight of tubers of all treatments of the first harvest, 21.62%, is lower than that of the second, 24.84%, and third harvests, 24.56%.

The time of harvest had a marked effect on both color and texture of the boiled tubers (Tables 3 and 4). At the first harvest there was only a faint trace of darkening in any of the samples regardless of planting date. At the second harvest, all samples showed more blackening than at the first harvest and at the final harvest the occurrence and intensity of blackening was greatest.

In general the texture ratings increased with the lateness of the harvest. Tubers grown at the four fertilizer treatments shown in Table 4 had an average texture rating of 3.75 at the first harvest, 7.17 at the second, and 8.08 at the third harvest. In general, the conditions which tend to produce Smooth Rural tubers which blacken after boiling also produce mealy tubers. These differences in texture and degree and extent of blackening, as will be shown later, are believed to be due mainly to the temperatures prevailing during a certain portion of the growing season.

TABLE I.—*Effect of time of planting, time of harvest, and fertilizer on specific gravity of tubers.*

Fertilizer treatment	First harvest, Aug. 16			Second harvest, Sept. 7			Third harvest, Oct. 5			Average
	Planted May 15	Planted June 1	Planted June 15	Planted May 15	Planted June 1	Planted June 15	Planted May 15	Planted June 1	Planted June 15	
None .....	1.094	1.087	1.070	1.095	1.098	1.095	1.095	1.097	1.098	1.092
5-10-0.....	1.092	1.086	1.066	1.096	1.096	1.094	1.100	1.099	1.097	1.092
5-10-5.....	1.094	1.086	1.066	1.095	1.095	1.094	1.095	1.090	1.089	1.089
5-10-10.....	1.094	1.080	1.063	1.096	1.093	1.094	1.096	1.094	1.094	1.090
5-20-10.....	1.092	1.080	1.064	1.094	1.094	1.098	1.096	1.094	1.093	1.089
0-10-10.....	1.091	1.081	1.070	1.094	1.092	1.093	1.093	1.088	1.090	1.088
10-10-10.....	1.090	1.075	1.068	1.097	1.094	1.098	1.093	1.092	1.093	1.089
5-10-10+3SDN*	1.092	1.073	1.065	1.095	1.091	1.092	1.097	1.090	1.088	1.087
5-10-10+3 SDK†	1.088	1.073	1.065	1.097	1.093	1.091	1.093	1.090	1.093	1.087
5-0-10.....	1.087	1.075	1.067	1.094	1.088	1.089	1.090	1.088	1.087	1.085
Average.....	1.091	1.079	1.066	1.095	1.093	1.094	1.095	1.092	1.092	—

\*SDN = 2% side dressing of nitrogen.

†SDK = 2% side dressing of potash.

TABLE 2.—*Effect of time of planting, time of harvest, and fertilizer application on percentage dry weight of tubers.*

Fertilizer treatment	First harvest, Aug. 16			Second harvest, Sept. 7			Third harvest, Oct. 5			Average
	Planted			Planted			Planted			
	May 15	June 1	June 15	May 15	June 1	June 15	May 15	June 1	June 15	
None.....	25.172	23.392	19.945	25.062	25.552	25.935	24.707	24.418	26.204	24.487
5-10-0.....	25.138	23.108	19.277	23.636	25.355	27.456	25.371	24.795	24.377	24.279
5-10-5.....	24.480	22.477	19.151	23.769	25.610	25.441	24.287	25.332	26.593	24.126
5-10-10.....	24.642	21.933	18.532	25.182	25.063	23.953	24.879	22.943	24.758	23.542
5-20-10.....	—	21.142	18.162	23.769	24.880	25.150	24.519	23.657	24.661	23.242
0-10-10.....	25.083	21.364	19.104	24.151	24.774	24.220	24.157	23.637	24.074	23.396
10-10-10.....	23.848	21.120	19.367	23.464	26.878	25.381	25.857	25.354	24.349	23.957
5-10-10+3 SDN*	24.931	20.264	18.059	24.554	25.046	24.258	24.273	23.798	23.283	23.163
5-10-10+3 SDK†	23.102	20.576	18.672	25.216	25.151	24.916	24.629	24.531	24.964	23.528
5-0-10.....	22.536	20.611	19.307	25.161	23.658	22.606	23.386	25.573	23.576	22.925
Average.....	24.326	21.598	18.957	24.396	25.196	24.931	24.606	24.403	24.684	—

\*SDN = 2% side dressing of nitrogen.

†SDK = 2% side dressing of potash.

TABLE 3.—*Ratings on blackening of tubers after boiling.\**

Fertilizer treatment	First harvest, Aug. 16			Second harvest, Sept. 7			Third harvest, Oct. 5		
	Planted May 15	Planted June 1	Planted June 15	Planted May 15	Planted June 1	Planted June 15	Planted May 15	Planted June 1	Planted June 15
None.....	—	—	—	—	9	—	5	8	7
5-10-0.....	9.5	9.5	9.5	9	—	—	8	8	7.5
5-10-5.....	—	—	—	—	—	—	9	6.5	7.5
5-10-10.....	—	—	—	—	—	—	8.5	5	5
5-20-10.....	—	—	—	—	—	—	6	5.5	4
0-10-10.....	—	—	—	8	7	7	8	5.5	5.5
10-10-10.....	9	9.5	10	—	—	—	8.5	6.5	7.5
5-10-10+3 SDN†	10	9.5	10	7.5	7.5	6.5	7.5	6.5	7
5-10-10+3 SDK‡	10	10	9.5	9.5	9.5	9	5	6	6.5
5-0-10.....	—	—	—	—	—	—	—	—	—
Average.....	9.6	9.6	9.7	8.5	8.2	7.9	7.5	6.25	6.3

\*10 = white one-half hour after boiling; 1 = extreme blackening one-half hour after boiling.

†SDN = 2% side dressing of potash.

‡SDK = 2% side dressing of potash.

TABLE 4.—*Ratings on texture of boiled tubers.\**

Fertilizer treatment	First harvest, Aug. 16			Second harvest, Sept. 7			Third harvest, Oct. 5		
	Planted May 15	Planted June 1	Planted June 15	Planted May 15	Planted June 1	Planted June 15	Planted May 15	Planted June 1	Planted June 15
None.....	6	4	—	8	8	—	9	9.5	10
5-10-0.....	—	—	2	—	—	7	10	9.5	9
5-10-5.....	—	—	—	—	—	—	9	7.5	7
5-10-10.....	—	—	—	—	—	—	10	9	10
5-20-10.....	—	—	—	—	—	—	10	9	9
0-10-10.....	6	4	3	8	7	7.5	8.5	7	7.5
10-10-10.....	—	—	—	—	—	—	8	8	8.5
5-10-10+3 SDN†.....	—	—	—	—	—	—	9.5	8	7
5-10-10+3 SDK‡.....	5	4	2	8	7.5	7	9	8.5	8.5
5-0-10.....	5	4	2	8	6.5	6.5	8	7.5	7
Average.....	5.5	4.0	2.25	8.0	7.25	7.0	9.1	8.35	8.35

\*10 = very mealy; 1 = very soggy.

†SDN = 2% side dressing of nitrogen.

‡SDK = 2% side dressing of potash.



## EFFECT OF FERTILIZERS

Tubers from unfertilized plots and from plots fertilized with 5-10-0 averaged higher in specific gravity than those from any of the other treatments. Tubers from the 5-0-10 plot were lowest in average specific gravity. Tubers from plots receiving 5-10-10 fertilizer and given three side dressings each of 2% potash or 2% nitrogen were also slightly lower than those of most of the other treatments. Similar results as to percentage dry weight of tubers were obtained. The ratings on texture correlated very closely with the specific gravity and dry weight of the tubers.

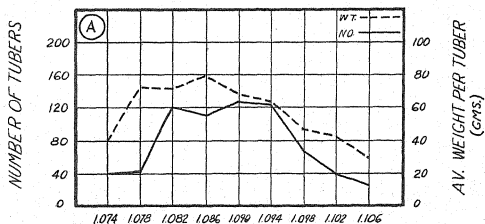
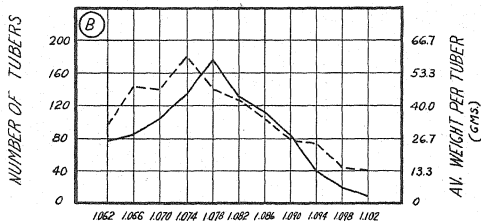
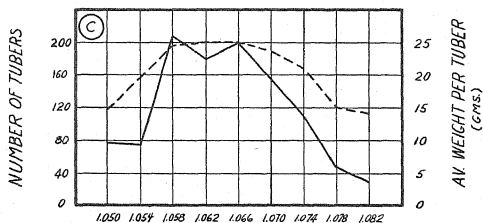
Practically no differences were noted in extent or intensity of darkening at the first harvest between the fertilizer treatments. However, at the second and third harvests, some differences were noted between the fertilizer treatments which resulted in advanced or retarded maturity. Those treatments which hastened maturity usually produced tubers which darkened less than those which retarded maturity. These differences in darkening undoubtedly were caused by differences in temperatures prevailing during the latter part of the growing period for each lot as will be shown later.

## RELATION OF SIZE OF TUBERS TO SPECIFIC GRAVITY

At the time specific gravity measurements were made the weight as well as the number of tubers in each of the specific gravity groups was recorded. In Figs. 1 to 4 are plotted the average weight per tuber and the number of tubers in each specific gravity group. Each figure represents the average weight per tuber or the number of tubers in each specific gravity group in all 10 fertilizer treatments of the planting and harvest indicated. In Fig. 4A the samples from all dates of planting and from all fertilizer treatments taken at the second harvest and representing a total of 2,601 tubers form the basis for the data plotted. In Fig. 4B similar samples from the last harvest were used and represent a total of 2,090 tubers.

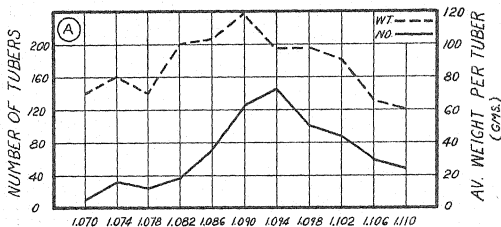
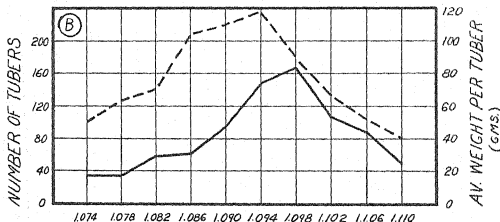
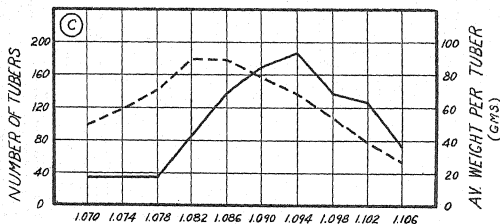
As noted previously (7), the number of tubers in each specific gravity group increases to a maximum and then steadily declines. However, the relation between size and specific gravity has not been formulated previously.

The results presented in Figs. 1 to 4 show that the average weight per tuber in each specific gravity range increased steadily to a maximum and then declined in much the same manner as did the number of tubers in each specific gravity group. Any explanation for this relationship must, at this time, be hypothetical. However, a logical explanation is based on three established principles as follows: (a) very immature tubers are lower in dry weight percentage than mature tubers, (b) small tubers contain a larger percentage of tissue outside the xylem than large tubers, and (c) the tissue outside the xylem is higher in percentage dry weight than the remainder of the tuber. Thus, the relatively small size of the tubers in the lowest specific gravity groups may be accounted for by the fact that they are the least mature and, therefore, are in the lower specific gravity groups because of the lower dry weight of immature tubers. On the other

1<sup>ST</sup> PLANTING-1<sup>ST</sup> HARVEST2<sup>ND</sup> PLANTING-1<sup>ST</sup> HARVEST3<sup>RD</sup> PLANTING-1<sup>ST</sup> HARVEST

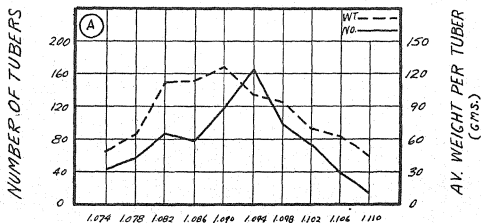
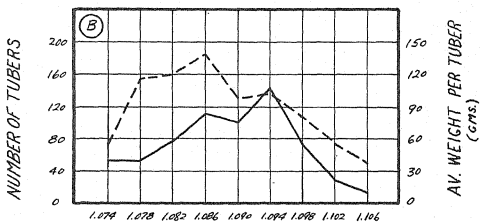
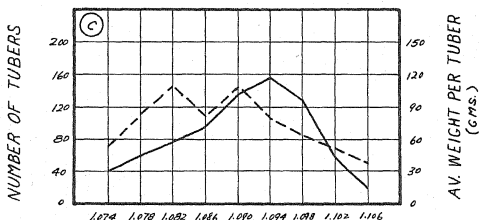
## SPECIFIC GRAVITY GROUPS

FIG. 1.—Number of tubers and average weight per tuber from first, second, and third plantings at first harvest.

1<sup>ST</sup> PLANTING-2<sup>ND</sup> HARVEST2<sup>ND</sup> PLANTING-2<sup>ND</sup> HARVEST3<sup>RD</sup> PLANTING-2<sup>ND</sup> HARVEST

## SPECIFIC GRAVITY GROUPS

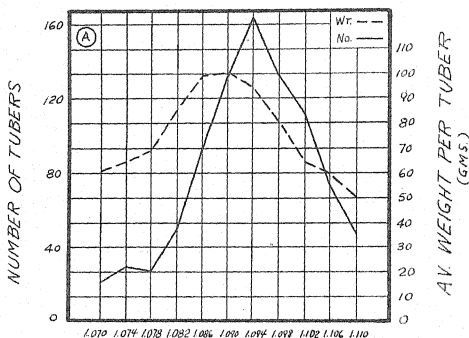
FIG. 2.—Number of tubers and average weight per tuber from first, second, and third plantings at second harvest.

1<sup>ST</sup> PLANTING-3<sup>RD</sup> HARVEST2<sup>ND</sup> PLANTING-3<sup>RD</sup> HARVEST3<sup>RD</sup> PLANTING-3<sup>RD</sup> HARVEST

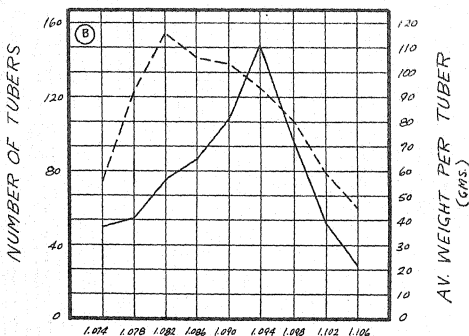
## SPECIFIC GRAVITY GROUPS

FIG. 3.—Number of tubers and average weight per tuber from first, second, and third plantings at third harvest.

TUBERS FROM THREE DATES OF PLANTING  
2<sup>ND</sup> HARVEST (2601 TUBERS)



TUBERS FROM THREE DATES OF PLANTING  
3<sup>RD</sup> HARVEST (2090)



SPECIFIC GRAVITY GROUPS

FIG. 4.—Number of tubers and average weight per tuber from three dates of planting at the second and third harvests.

hand, one may account for the small size of the tubers in the high specific gravity groups on the basis that they are mature or more nearly mature than the small tubers in the low specific gravity groups and are high in specific gravity because of the relatively greater proportion of tissue outside the xylem than there is in the larger tubers.

Another significant point is that the average weight per tuber reaches a maximum in a lower specific gravity group than does the total number of tubers. At the last harvest the maximum weight per tuber was reached in the 1.082 group, whereas the greatest number of tubers were concentrated in the 1.094 group (Fig. 4B). A possible explanation for this is that the largest mature tubers would contain relatively less tissue outside the xylem than the slightly smaller tubers and, therefore, be lower in specific gravity. Tubers of average size for any given sample would be present in greatest numbers and would contain a relatively greater amount of tissue outside the xylem than would larger tubers and, therefore, would be of higher specific gravity than tubers of larger sizes.

### DISCUSSION

Since some of the results of several of our other experiments have indicated that light and temperature exert considerable influence on resultant texture and color of boiled potatoes, it was thought that some of the differences in quality observed in these experiments could be explained on the basis of differences in these environmental factors. In Table 5 are presented average maximum and minimum temperatures and approximate percentage of sunshine for periods previous to each of the three harvests. All vines were dead by October 1; therefore, temperature and light conditions after that date are not included.

TABLE 5.—Average maximum and minimum temperatures and approximate percentage of sunshine.

Period	Average maximum temperature, °F*	Average minimum temperature, °F*	Approximate percentage sunshine†
Aug. 1-15.....	74°	52°	70
Aug. 16-Sept. 6.....	64°	46°	55
Sept. 7-Oct. 1.....	60°	39°	49

\*Data obtained on farm where tubers were grown, Cohocton, N. Y.

†Data obtained at Ithaca, N. Y.

As the temperature became lower and therefore more favorable for the production of tubers of high dry weight, high specific gravity, and greater mealiness, light conditions were becoming less favorable for these factors by an increase in cloudiness. Tubers planted earliest and harvested August 16 while they were still quite immature were practically as high in specific gravity and dry weight as those from any other treatment. They were, however, rated much less mealy than those from later harvests.

At the first harvest practically no blackening was observed in any of the samples. Relatively high temperatures and intense light condi-

tions prevailed previous to this harvest and thus lend further evidence that these environmental factors are of utmost importance in decreasing or preventing blackening. The degree and extent of blackening in most samples became increasingly greater with the two later harvests, thus paralleling the increasing unfavorableness of lower light intensity and lower temperature conditions.

Although there were wide variations in fertilizer treatments, there were few consistent differences in dry weight, specific gravity, texture, or color of boiled potatoes induced by fertilizers. The differences which did appear seem to be due to the effects of the fertilizer on hastening or retarding maturity and, therefore, subjecting them to different environmental conditions during the latter part of the growing season. This would lead one to believe that environmental factors other than nutrition are of major importance in determining cooking quality of potatoes.

#### SUMMARY

Smooth Rural potatoes subjected to 10 different fertilizer treatments were planted at three different dates and potatoes of each of these treatments were harvested on three different dates. Thus, tubers were obtained which were of different degrees of maturity and which during the latter portion of their growing period had been subjected to different light and temperature conditions. Tubers were used for specific gravity measurements, dry weight determinations, and cooking tests for determination of color and texture differences.

Tubers at the first harvest from the first planting were consistently higher in specific gravity and dry weight than those from the second and third plantings.

The time of planting had no consistent effects on the occurrence of blackening. The more mature tubers were of a more mealy texture than those planted later and less mature.

The specific gravity of tubers of the second and third plantings at the first harvest is consistently lower than those of the same dates of planting at the second and third harvests. Similar differences appear in dry weight of tubers of the three harvests.

The time of harvest had a marked effect on color and texture of boiled tubers. The later harvested tubers blackened to a greater extent and had a higher texture rating than those harvested early.

Relatively small differences exist in specific gravity and dry weight of tubers grown with the various fertilizer treatments. Differences in degree and extent of darkening which occurred were related to the effect of the fertilizer on hastening or retarding maturity.

The average weight per tuber as well as the number of tubers in each specific gravity group increased steadily from the low range to a maximum at the medium range and then declined at the high specific gravity range.

A relationship exists between temperature and light conditions during the latter part of the growing season and the specific gravity, dry weight, color, and texture of boiled tubers grown under those conditions.

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## INHERITANCE OF SEED COLOR IN BIENNIAL WHITE SWEET CLOVER, *MELILOTUS ALBA*<sup>1</sup>

S. P. SWENSON<sup>2</sup>

IN a previous paper, Fowlds (4)<sup>3</sup> has referred to a number of inherited seed color variations which have been observed and reported in sweet clover. Among those which he cited were two which he found and isolated, green and pale yellow. His investigations revealed that normal yellow seeds have yellow seed coats and yellow cotyledons, green seeds possess green seed coats and green cotyledons, and pale yellow seeds, colorless seed coats and yellow cotyledons.

From an inheritance study, Fowlds found that green when crossed with yellow behaved as a simple recessive and that the same gene pair determined color in both the seed coat and cotyledons. Two types of pale green seeds occurred; one with yellow seed coat and green cotyledons, the other with green seed coat and yellow cotyledons.

The results from a limited number of  $F_2$  plants of the cross pale yellow  $\times$  yellow suggested a 3:1 ratio with pale yellow recessive to yellow. In this cross, only the color in the seed coat was affected, all cotyledons being yellow.

Fowlds studied the green  $\times$  pale yellow cross only as far as  $F_1$ . The  $F_1$  plants were found to produce yellow seed coats but segregated as expected for yellow and green cotyledons. The results suggested complementary factors necessary for color production in the seed coat, perhaps a basic factor for color production, and a dominant factor for yellow.

It is the purpose of this paper to present the results obtained from  $F_2$  plants of the cross, green  $\times$  pale-yellow, with special reference to the inheritance of seed color. Since the green-seeded parent has a dwarf habit of growth as contrasted to the normal habit of the pale-yellow parent, data on the inheritance of growth habit are reported. Some observations on the incidence of a root rot also have been included.

### MATERIALS AND METHODS

In February 1938, a plant homozygous for green seed and dwarf habit of growth was crossed with a plant homozygous for pale yellow seed and normal habit of growth, both parents having been selected from selfed lines. The  $F_1$  seedlings were grown in the field during the summer and nine of the plants were removed to the greenhouse in November. Selfed seed was obtained from these plants in March 1939 and separated into two classes for cotyledon color, yellow and green. The yellow and green seeds were planted separately in the greenhouse and the two groups of seedlings were later set out and spaced in separate plots in the field.

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<sup>2</sup>Formerly Associate Agronomist. The author acknowledges his indebtedness to Matthew Fowlds, formerly Assistant Agronomist, for the material used in this study.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 459.

Immediately before blossoming in 1940, two racemes on each plant were covered with cloth bags for selfing and allowed to mature. At the time of harvesting the selfed racemes, open-pollinated racemes also were harvested from each plant so that if selfed seed was not obtained, the genotype of each plant might still be determined for cotyledon color.

The seeds were later stripped from the racemes and rubbed out of the hulls between canvas-faced wooden blocks. If the selfed racemes of a plant yielded no seed or an insufficient number of seeds, enough of the open-pollinated seed was threshed to provide for a determination of the genotype for cotyledon color. The selfed or open-pollinated seeds of each plant were then classified into one or more of four classes, yellow, pale yellow, pale green, and green. Improperly developed seed was not included in the classifications.

During the growing season of 1940, the growth habit of each plant was recorded as normal, intermediate, or dwarf. In addition, the actual height in inches of each plant was measured and recorded.

Because of a severe infestation of root rot,<sup>4</sup> each plant was classified into one of six classes for severity of infection, 0, trace, light, medium, heavy, and dead. In addition, the number of plants completely missing from the previous year was determined and recorded.

The  $\chi^2$  tests for goodness of fit and independence were employed in analyzing the results. Fisher's (3) table of  $\chi^2$  was used for obtaining values of P.

## EXPERIMENTAL RESULTS

### INHERITANCE OF SEED COLOR

In analyzing the results of the classification for seed color in  $F_2$  plants, it should be noted that the seed coat is  $F_2$  tissue while cotyledon color exhibits an  $F_3$  segregation. The apparent color of the seed is therefore the result of the combination of colors present in the two parts of the seed.

In the group of  $F_2$  plants grown from  $F_1$  seeds having yellow cotyledons, four types of plants occurred as follows: (a) Segregating for yellow and pale green, (b) all yellow, (c) segregating for pale yellow and green, and (d) all pale yellow. Selfed seeds of plants grown from  $F_1$  seeds having green cotyledons were all green.

If the hypothesis suggested by Fowlds (4) is used, namely, that the pale yellow parent lacks a basic dominant gene necessary for color production in the seed coat, then the arrangement shown in Table 1 should explain the results. The basic gene for color has been designated as  $C$ , its allele as  $c$ , and the gene pair for yellow versus green as  $Yy$ , the symbols used by Fowlds (4).

Because the yellow- and green-seeded groups were grown separately rather than as one large random sample, they will be treated separately. One hundred and forty plants from yellow  $F_1$  seeds were available for selfing. Of this number, 101, or 72.1%, of the plants set seed, but several of these plants produced too few selfed seeds for

<sup>4</sup>Probably root-rot caused by *Phytophthora cactorum*, a rather common disease in space-planted sweet clover nurseries. The writer acknowledges receipt of information on this disease from members of the sweet clover conference held in conjunction with the annual meeting of the American Society of Agronomy at Chicago in December, 1940 (6).

TABLE 1.—*The possible genotypes and phenotypes, and their expected frequencies, of F<sub>2</sub> plants from the cross green(Ccy)<sup>1</sup>—X pale yellow(ccY)<sup>2</sup>—seeded sweet clover.*

Genotype	Expected frequency	F <sub>1</sub> cotyledon*	Appearance of F <sub>1</sub> seed†	F <sub>2</sub> seed coat‡	F <sub>2</sub> cotyledon	Appearance of F <sub>2</sub> seed§
CCYY.....	1	Yellow	Yellow	Yellow	All yellow	All yellow
CCYy.....	2	Yellow	Yellow	Yellow	3 yellow:1 green	3 yellow:1 pale green
CcYY.....	2	Yellow	Yellow	Yellow	All yellow	All yellow
CcYy.....	4	Yellow	Yellow	Yellow	3 yellow:1 green	3 yellow:1 pale green
CCyy.....	1	Green	Pale green	Green	All green	All green
Ccyy.....	2	Green	Pale green	Green	All green	All green
ccYY.....	1	Yellow	Yellow	Colorless	All yellow	All pale yellow
ccYy.....	2	Yellow	Yellow	Colorless	3 yellow:1 green	3 pale yellow:1 green
ccyy.....	1	Green	Pale green	Colorless	All green	All green

\*3 yellow:1 green.

†All F<sub>1</sub> seed has a yellow seed coat.

‡3 yellow:3 green:4 colorless.

§3 all yellow: 6 segregating (3 yellow:1 pale green):1 all pale yellow:2 segregating (3 pale yellow:1 green):4 all green.

deciding whether or not segregation for cotyledon color was occurring.<sup>5</sup> For such plants and the remaining 39 from which no selfed seed was obtained, open-pollinated seed was used to determine the presence or absence of segregation. The four classes of plants and their respective frequencies are shown in the first four columns of Table 2. On the basis of the hypothesis shown in Table 1, the four classes should occur in a ratio of 6:3:2:1. It may be seen from Table 2 that the observed frequencies are in good agreement with the calculated, as indicated by the  $\chi^2$  test.

TABLE 2.—*The descriptions and the observed and calculated frequencies of the four classes of  $F_2$  plants grown from  $F_1$  seeds having yellow cotyledons.*

$F_2$ seed coat	$F_3$ cotyledons	Appearance of $F_2$ seed	Observed frequencies	Calculated frequencies
Yellow . . . . .	Yellow and green	Yellow and pale green	78	70.00
Yellow . . . . .	All yellow	All yellow	26	35.00
Colorless . . . .	Yellow and green	Pale yellow and green	25	23.33
Colorless . . . .	All yellow	All pale yellow	11	11.67

$$\chi^2 = 3.386; P = .50-.30.$$

Seventeen, or 63.0%, of 27 selfed plants having green cotyledons set selfed seed under the bags. No cross-pollination from nearby yellow plants was observed in any of the 17 selfed samples, but examination of the open-pollinated seed from each plant revealed that every plant had been cross-pollinated to some extent with pollen carrying the gene for yellow cotyledon. Seed resulting from such cross pollination served as an excellent means of distinguishing between plants having green seed coats and those having colorless seed coats in that the former had pale green and the latter pale yellow seeds. Consequently, it was unnecessary to separate the seed coats from the cotyledons in order to determine presence or absence of color in the seed coat. The two expected classes with the observed and expected frequencies are presented in Table 3. The agreement of the observed with the expected 3:1 ratio is very good.

TABLE 3.—*The descriptions and the observed and calculated frequencies of the two classes of  $F_2$  plants grown from  $F_1$  seeds having green cotyledons.*

$F_2$ seed coat	$F_3$ cotyledons	Appearance of $F_2$ seed	Observed frequencies	Calculated frequencies
Green . . . . .	Green	Green	23	20.25
Colorless . . . .	Green	Green	4	6.75

$$\chi^2 = 1.494; P = .30-.20.$$

Further information on the inheritance of yellow versus green cotyledons is available from the first and third classes in Table 2 which are segregating for the gene pair  $Yy$ . It is evident from the data

<sup>5</sup>Failure to set seed is not necessarily an indication of self-sterility in this study. A severe rain and wind storm broke off some of the bagged racemes and may have interfered with seed setting in other instances.

shown in Table 4, particularly from the plants having yellow seed coats, that there is a tendency toward an excess of green and a corresponding deficiency of yellow seeds. A careful examination of a number of the samples revealed that slightly immature yellow seed coats tend to retain considerable green color and are therefore difficult to classify. Fowlds (4) encountered similar difficulty in his classification. Colorless seed coats, on the other hand, permit a more accurate classification for cotyledon color as indicated by the  $\chi^2$  tests in Table 4.

TABLE 4.—*The observed frequencies of yellow and green cotyledons within the two classes for seed coat color, yellow and colorless, with  $\chi^2$  values on the basis of a 3:1 ratio.*

Seed coat class	Cotyledon color		$\chi^2$	P
	Yellow	Green		
Yellow.....	6,145	2,209	9.270	.01
Colorless.....	2,112	728	.608	.50-.30
Total.....	8,257	2,937	9.139	.01

Among the selfed plants segregating for yellow and green cotyledons, 52 having yellow seed coats and 15 having colorless seed coats set sufficient numbers of seeds to permit the calculation of individual  $\chi^2$  values for each plant. Addition of the 67  $\chi^2$  values resulted in a total of 98.828 which when substituted in the formula  $\sqrt{2\chi^2 - \sqrt{2N} - 1}$  for  $N=67$  gave a value of 2.53. In Fisher's (3) "t" table, this corresponds to a P value of slightly above .01, indicating that the fit of the observed to the expected frequencies is not very satisfactory.

The 67  $\chi^2$  values also were grouped into a frequency distribution and compared with the expected distribution, according to the method of Kirk and Immer (8), resulting in  $\chi^2$  and P values of 15.163 and .02 to .01, respectively. Seven of the 67 P values fell below .05. On examining the data, six of the seven progenies showed excesses of green and deficiencies of yellow seeds, even after attempts were made to reclassify the seeds. It was noted, however, that the seed in most of these samples appeared to be slightly immature but otherwise well developed. Consequently, part of the discrepancy very probably could be due to errors in classification.

In view of the difficulty encountered in classification of some progenies, the evidence still appears to be in favor of a single gene pair for yellow versus green color in both the cotyledons and seed coat. Under the conditions of the study, the assumption of the gene *I* causing green pigment to fade at maturity, as suggested by Hartwig (5), appears to be unnecessary. In the case of seed coat color, when all the colored are considered against all the colorless, 127 colored and 40 colorless  $F_2$  plants were obtained. Tested against a 3:1 ratio, a  $\chi^2$  value of .098 and a P value of .80-.70 were obtained, demonstrating a very good fit of observed to the expected.

Independence of the gene pairs  $Cc$  and  $Yy$  already has been indicated by the  $\chi^2$  test for goodness of fit in Table 2. When tested for independence,  $\chi^2$  and  $P$  were 1.476 and .30-.20, respectively, definitely indicating independent inheritance of the two gene pairs.

The logical conclusion is that the occurrence of yellow, pale yellow, green, and pale green seeds in sweet clover may be explained on the basis of two independent gene pairs,  $Cc$  and  $Yy$ . The presence of  $C$  is necessary for color production in the seed coat; if absent, lack of color results.  $Y$  produces yellow color in the cotyledons and also in the seed coat in the presence of  $C$ , while  $y$  produces green color in the cotyledons and also in the seed coat if  $C$  is present.

#### INHERITANCE OF GROWTH HABIT

The original green-seeded selection had a dwarf habit of growth, similar to the Alpha types of Kirk (7) and Stevenson (9), or perhaps the dwarf types of Clarke (1) and Elders (2). Subsequent selections, including the parent used in this study, retained this growth habit.  $F_1$  plants of the cross, green  $\times$  pale yellow (dwarf  $\times$  normal), displayed an intermediate habit of growth. Three classes were therefore recognized in the  $F_2$ , normal, intermediate, and dwarf. With only a few exceptions, the height measurements were in good agreement with the categorical classification. In some instances, however, severe root-rot infection reduced the size of a plant to a point where it might be placed in a lower category. Since leaf size and general plant texture also serve to identify the three classes for growth habit, the categorical classification has been used because it appears to be a more accurate criterion of growth habit than height only.

The data presented in Table 5 show an acceptable agreement between the observed and calculated frequencies for the three classes on the basis of a 1:2:1 ratio. The mean heights in inches of the three classes also are presented to show actual differences in height. There is a tendency toward an excess of intermediate types at the expense of normal types, perhaps because of errors in classification due to root-rot infection. Since  $F_3$  progeny tests have not been made, the evidence cannot be considered as fully conclusive.

TABLE 5.—*The observed and calculated frequencies of 167  $F_2$  plants grouped into three classes according to growth habit.*

Growth habit	O	C	Mean height in inches
Normal tall.....	31	41.75	56.6
Intermediate.....	92	83.50	36.6
Dwarf.....	44	41.75	22.0

$\chi^2 = 3.754$ ;  $P = .20-.10$ .

Tests were made for independence of the gene for growth habit and the two genes determining seed color. The  $\chi^2$  and  $P$  values shown in Table 6 indicate independence in both instances.

TABLE 6.—*Tests for independence of the gene for habit of growth and the two genes determining seed color, as determined from 2×3 tables with 2 degrees of freedom for 167 F<sub>2</sub> plants.*

Items	$\chi^2$	P
Growth habit and yellow vs. green.....	.650	.80-.70
Growth habit and colored vs. colorless.....	2.409	.30

## OBSERVATIONS ON ROOT-ROT

A total of 496 plants were set out in 1939. Practically all of these plants survived the summer of 1939 in good condition. Early in the spring of 1940, 299 plants were dead or missing, most of them visible but dead or nearly dead. Of the remaining 197, 30 died before blossoming, leaving 167 plants available for bagging. Fourteen of the 167 died prematurely after bagging but set some seed before dying. The cause of the dead and diseased plants appeared to be the root-rot, previously mentioned (6). Among the remaining 153 plants, various degrees of infection were noted, from none to heavy infection. The results are summarized in Table 7. Whether actual inherent differences exist among the plants in resistance or susceptibility cannot, of course, be definitely determined without a progeny test.

TABLE 7.—*The distribution of F<sub>2</sub> plants according to severity of infection with root-rot.*

0	Trace	Light	Medium	Heavy
72	16	30	19	16
Died after blossoming	Died before blossoming	Missing plants	Total	
14	30	299	496	

$\chi^2$  tests indicated independence between root-rot infection and the genes for seed color and association between root-rot and growth habit. It is questionable, however, whether dwarf or intermediate plants are actually more susceptible or if they were classified as dwarfs or intermediates because of stunting by the disease. Unfortunately, no data were obtained on the relative susceptibility of the two parents.

## SUMMARY

The occurrence of four seed colors in biennial white sweet clover, normal yellow, pale yellow, pale green, and green, has been shown to be attributable to various combinations of two independent gene pairs, *Cc* and *Yy*. *C* is necessary for color production in the seed coat, *cc* plants having colorless seed coats. *Y* and *y* produce yellow and green cotyledons, respectively, regardless of seed coat color; however, in the presence of *C*, yellow or green color also develops in the seed coat. Seeds with colorless seed coats and yellow cotyledons (*ccY*) are

pale yellow; those with colorless seed coats and green cotyledons (*cy*) cannot be identified from normal green by ordinary visual examination.

Normal and dwarf habit of growth in the cross green  $\times$  pale yellow are differentiated by a single gene pair, the heterozygotes being intermediate in growth habit. The gene for growth habit was independent of the two genes determining seed color.

A severe infestation of root-rot resulted in a loss of two-thirds of the plants originally set out and appreciable dying and stunting among the remaining plants. There was some evidence to indicate that dwarf and intermediate plants were more susceptible than normal tall plants, but the data are inconclusive.

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## THE LOSS OF NODULES FROM LEGUME ROOTS AND ITS SIGNIFICANCE<sup>1</sup>

J. K. WILSON<sup>2</sup>

NODULES on the roots of legumes appear and develop as the plant expands its leaves and approaches the production of seed. They appear to remain on the plant or on the stubble as long as their metabolism harmonizes with that of the plant and are lost apparently only when the plant or its root system is placed under adverse conditions. They derive carbohydrates from, must be nourished by, and demand other relations with the plant if they are to survive. When one or more of these physiological relations is disturbed, some of the nodules cease to perform any useful function, use up their stores, reach a senile condition, decompose through the activity of autolytic enzymes and microorganisms, and liberate their nitrogen and other constituents in the soil.

The content of nitrogen of nodules is usually much higher than that of any other part of the plant. According to Joshi (4),<sup>3</sup> the content of nitrogen of the nodules of San-Hemp one week old was 10.81% of the dry weight, while that of nodules 6 weeks old was only 3.36%. It appeared possible that decomposing nodules have dissipated a portion of their nitrogen before visible detection of decomposition is evident. If nodules with varying contents of nitrogen are shed, they should behave like decomposing organic matter in the soil. Evidence that organic matter is available from plant roots is provided by the fact that the highest population of organisms in the soil capable of decomposing plant residues is found near the roots. Since the nodules are rich in nitrogen and decompose readily, the nitrogen they contain should be available or become available to any plant or organism growing on or in the soil.

Observations by Wilson (11) indicate that a reduction in the moisture content of a soil caused a shedding of nodules from *Phaseolus* and that new nodules succeeded the lost ones after the original content of moisture was restored. Wilson suggested that this alternate shedding of nodules and the development of new ones might occur several times during the growing period of the plant if moisture relations were changed. Each time the shedding occurred the nitrogen in the shed nodules was liberated in the soil. Conditions other than a lowering of the moisture may produce the same effect according to Giltner (2) and Leonard (5). Thus, environmental factors may cause the liberation and circulation of the nitrogen fixed in the nodules during the growing period of the plant.

Experiments by Lyon and Bizzell (7) showed that timothy grown in association with alfalfa, and oats grown either with peas or clover contained a higher content of nitrogen than when grown alone. The explanation of these findings was that the roots of these legumes

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 470.

excreted or sloughed off organic matter which contained nitrogen that was subsequently taken up by the nonlegume.

Experiments by Lipman (6) gave similar results. He suggested that the nitrogen was excreted by the leguminous plant and that it was taken up by the nonlegume.

Westgate and Oakley (10) pointed out that certain nonlegumes were benefitted by growing in association with a certain legume, while others were not. The suggested mechanisms and explanations given by workers to account for the passing of the nitrogen from the nodule of the legume during its development to the nonleguminous plant have been reviewed and summarized by Wilson (12).

It appears also from the work of Lyon and Bizzell (8) that as much as 297 pounds of nitrogen an acre was fixed annually where the soil was occupied by alfalfa. Data presented by Johnstone-Wallace (3) show that 12 cuttings in one season of Kentucky bluegrass (*Poa pratensis*) and wild white clover (*Trifolium repens*) growing in association removed 309 pounds of nitrogen. His results were compared with data from an application of 44 pounds of nitrogen an acre in the form of calcium cyanamide, sulfate of ammonia, or nitrate of soda in which only the latter gave a small increase in yield. Such results he considers as an indication of the effectiveness of wild white clover in supplying the nitrogen required for pasture production. Other workers have obtained data somewhat similar to those given by Johnstone-Wallace. These are reviewed by Wilson (12).

From such data one is stimulated to seek additional information concerning what happens physiologically to the nodules of leguminous plants after clipping or after shock from other causes which makes it possible for the nitrogen that is fixed symbiotically to be released in the soil and thus become available during the growing season to an associating nonlegume. It is hoped that the information detailed in this paper can be used for that purpose.

## METHODS

Since certain environmental factors when imposed on nodulated plants cause them to shed their nodules, it was thought that frequent clipping or defoliation of plants similar to that which plants received in the field where nitrogen becomes available to the nonlegume might be one of these. In this case the nitrogen in the shed nodules would be thrown out in the soil and thus be utilized by any plant or organism. To make such observations, 1,200 grams of Dunkirk fine sandy loam were placed in small glazed crocks. These were seeded with an equal quantity of wild white clover and 26 ml of water added for each 100 grams of the air-dry soil. The crocks were then placed in a water bath in the greenhouse. This was on June 26, 1941. A little water was allowed to run into the bath constantly and an overflow was provided. This maintained a temperature suitable for a slow growth and was the same for all crocks.

This temperature was imposed in order to lower the physiological activities of the nodules and organisms in the soil and thus make it possible to follow more closely the nodular changes effected by clipping. At the conclusion of the tests it was estimated that it required about 40 days in the water bath to effect the same degree of decomposition as was usually obtained in about 10 days in the field in

June or July. Moisture was maintained by daily weighings and the addition of water. Attempts were made to prevent a loss greater than 10%, although slightly more than this was lost once in the early stage of growth. No allowance was made for the increasing weight of the crop. When the plants obtained the desirable height, which in this case was about 4 inches, the tops were clipped to within  $\frac{1}{2}$  inch of the surface of the soil. Periodically, to obtain information, the plants in a crock were washed and their roots examined for the condition of the nodules which they bore. Nitrogen was determined by the Kjeldahl method.

#### PRESENTATION OF DATA

Although most of the data reported in this paper were obtained from plants of wild white clover, other data were accumulated by scrutinizing the root systems and nodules of plants from several other genera.

##### YOUNG AND SENILE NODULES ON CLOVER ROOTS GROWN IN GREENHOUSE

At the first cutting, when the white clover seedling was about 7 weeks old, the nodules were in excellent condition. No soft ones were found and each one appeared to be functioning perfectly. Ten days after the first defoliation, nodules were found that appeared to be disintegrating. They had lost some of their pinkish or greenish color and looked waterlogged. Such a condition was more marked after 25 days, and after 45 days many nodules were in an advanced stage of complete disintegration. On a few plants nearly all of the nodules were disintegrating, while on many others only sound-appearing nodules were present. From observation and counts it was estimated that about 15% of all nodules were visibly affected by clipping. Nodules that should have been present on the upper 1 inch of the tap root of most plants could not be found 42 days after the first clipping and 15 days after the second clipping. In some cases the nodules near the crown were collapsed, lying closely against the root, as shown in Fig. 2. Plants clipped only once and examined 15 to 25 days later sometimes revealed these collapsed nodules on various parts of the root system. When such nodules were removed they consisted apparently of the cortical shell of the nodule from which the parenchymatous tissue had disappeared. Such hulls were found more often on the main root near the crown, although some were found further down on the tap root and on the smaller roots also. When immersed in water and examined under the microscope, many of these nodules in the advanced stage of disintegration contained bubbles of gas.

At the first clipping a few plants had crowns a considerable distance above the soil. By clipping to  $\frac{1}{2}$  inch these were removed. The remaining portion consisting of the roots did not produce new growth and the nodules on these were also in the process of decay. They resembled in every way those that were decomposing on plants which were sending out new top growth.

Plants reaching a height of 4 inches the second time were harvested on September 2 and their roots examined. On many small rootlets

tiny nodules were appearing. On some plants six or eight were present. From their size, location, and whitish color, it was judged that they were succeeding those which had been shed and those on the plants which were about to be shed. Very few new nodules were found on plants that had not lost them from near the crown or in positions near the surface of the soil. Such observations coincide with those reported by Wilson (11) in which nodules were shed by bean plants after a reduction in moisture and a new lot restored after reestablishing the original moisture condition.

EFFECT OF CLIPPING ON POSITION OF NODULES ON CLOVER  
GROWN IN GREENHOUSE

When white clover plants were grown from seed to a height of about 4 inches, the largest volume of nodules as determined by inspection was found on the tap root near the crown of the plant. Many such nodules were within  $\frac{1}{2}$  inch of the surface of the soil. They apparently represented the first ones to appear on the seedlings. After clipping of the plants these nodules very largely disappeared and new ones appeared further down, either on the main root or on the laterals. If the plants produced runners and these developed roots, new nodules were produced on these. Such a shift in the position of the nodules, apparently due to clipping, can be visualized by comparing Figs. 1 and 2. It will be noted on comparing these two illustrations that not only have the nodules disappeared from the roots near the crown of the plants, but many of the lateral roots have disappeared also.

Although counts of nodules were made, it was difficult to establish differences because of the newly formed nodules. One fact was evident, however. The average number of nodules on the roots of plants clipped twice was about 16, while the average on the roots of those clipped once was more than 22, yet the seeding date for both was the same. Such averages were obtained by counting all plants and all nodules from each of four crocks. The plants numbered for each crock about 300 and the nodules 4,500. The difference between the averages of the nodules per plant from each crock was less

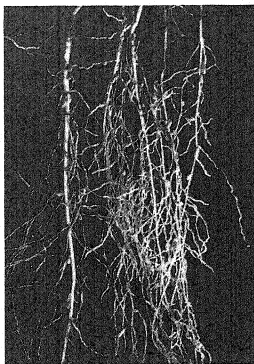


FIG. 1.—Roots of wild white clover bearing nodules. Plants clipped when 7 weeks old and roots photographed 25 days later. Nodules are still on tap root near crown of plant but most of them appear waterlogged and to be disintegrating. Others are so transparent that they offer little resistance to the light. Compare with Figs. 2 and 3.

than 1. If one could differentiate accurately between the nodules formed on seedlings and those formed after clipping, it is evident that a wider difference would have been found.



FIG. 2.—Roots of wild white clover bearing nodules. Plants clipped when 7 weeks old. Roots photographed 42 days later, which was 15 days after second clipping. Nodules have been shed principally from upper part of tap root. Numerous nodules still on roots are in the process of disintegration. There are 14 such nodules on the seventh plant from the left. Tiny nodules are appearing on the fibrous roots. (Sept. 8, 1941.)

In one case the plants in a crock were clipped only once and the new growth allowed to reach a height of about 5 inches. This took about 42 days. The roots were then examined for the location and condition of the nodules. It was observed that many nodules on the tap root near the surface of the soil still remained. Some of these, however, were in the process of decay. One plant from this crock bore 20 nodules in this advanced stage of decomposition, another 22, and still another 25. Such partly decomposed nodules can be seen in Figs. 2 and 3. It was apparent that a single clipping did not cause such a

drastic shedding of the nodules as did two clippings, although the seedlings were of similar age. The effect of two clippings over one clipping on the shedding of nodules and on the extent of the roots can be seen in Figs. 2 and 3.

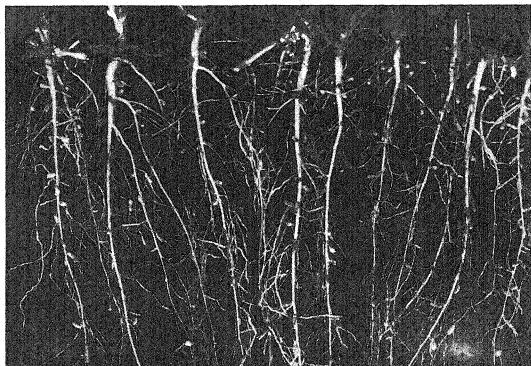


FIG. 3.—Roots of wild white clover bearing nodules. Plants clipped when 7 weeks old. Roots photographed 42 days later. Nodules on upper part of tap root have not completely shed, although some appear partly decomposed. The second plant from the left had 22 in this condition, the third 25, and the fourth 20. They are the darker appearing nodules.

The observed condition of the nodules after clipping, together with this difference in the position of the nodules and the appearance of new ones, are taken as evidence that clipping caused a shedding of some of the nodules. Since nodules were not present on the roots of clipped plants where it was evident they should have been and since no evidence of them could be found in the soil, it was presumed that they were completely disintegrated and that their content of nitrogen and other constituents had been absorbed or liberated in the soil.

#### MICROSCOPIC APPEARANCE OF CONTENTS OF YOUNG AND OF SENILE NODULES FROM CLOVER GROWN IN GREENHOUSE

Further evidence was obtained concerning the shedding and decomposition of nodules from white clover. Sound nodules and those that appeared to be in various stages of decomposition were examined microscopically. To do this the two kinds of nodules were thoroughly washed and then crushed on microscopic slides. After fixing by heating, the preparations were stained with carbol-fuchsin and examined. Many typical pear-shaped bacteroids and short rods with rounded ends, typical of the nodule bacteria, were present in the preparation

of the firm nodule. Those of the nodules in an advanced stage of decomposition gave an entirely different picture. The population was heterogeneous and no bacteroid forms were seen. The material appeared much like decomposing organic matter. It was not difficult to differentiate microscopically between the two types of nodules.

#### YOUNG AND SENILE NODULES ON CLOVER GROWN IN THE FIELD

It appeared advisable to observe the condition of the nodules on wild white clover growing in the field. Plats were selected where the clover was being clipped each time it was about 4 inches high. Sods were taken from these plats and brought to the laboratory. The plants, although in bloom, were composed largely of runners whose nodes had sent roots into the soil. These roots were carefully washed and the nodules examined. Firm or sound nodules were present and nodules in many stages of decomposition were found. These, however, appeared to comprise only about 5 to 8% of the nodules.

#### ABSENCE OF NODULE BACTERIA IN SENILE NODULES OF CLOVER GROWN IN THE FIELD

The waterlogged appearance of numerous nodules and the presence of bubbles of gas in others, together with the type of organisms distinguishable under the microscope, suggested that it would be difficult, if not impossible, to isolate the legume bacteria from the soft and hull-like nodules, while, on the other hand, it would be comparatively easy to do so from the turgid or firm ones. Accordingly, nodules of both types were selected and placed in a filtered 1% solution of calcium hypochlorite for 10 minutes, then washed in sterile water containing 0.05%  $\text{KH}_2\text{PO}_4$ , and after another 10 minutes crushed in sterile petri dishes. An agar medium suitable for the germs was then poured in the dishes and these held at room temperature to allow the development of colonies. The result was clear cut.

Four sound nodules and four in an advanced stage of decomposition were plated individually. From inspection of colonies and from stained mounts, it was judged that the colonies appearing on the plates where the four sound nodules were used were predominantly nodule bacteria, while those developing on plates where the decomposing nodules were used were predominantly something else. It was questionable whether any of these latter colonies consisted of legume bacteria. They certainly were not colonies that would be selected for further tests or use unless nothing better was available.

#### YOUNG AND SENILE NODULES ON OTHER LEGUMES GROWN IN THE FIELD

*Pea*.—Supplementing these data from wild white clover are the results from Canada field pea (*Pisum sativum*). The crop was seeded in the open field and grew for a time while the moisture content of the soil was fairly high. When the plants were about 6 to 8 inches tall, the soil became exceedingly dry and plant growth was retarded. After a rain when active growth was resumed, the roots of the peas were ex-

aminated. Pits or scars were found on the roots from which nodules had been sloughed. In some cases the rim of the pit was fringed with remnants of tissue resembling that of nodules. Also in some cases only the outer shell of the nodule was found, the inner part having disintegrated. In several instances the nodules, partly decomposed, were found floating on the water in which the roots were being washed.

*Birdsfoot trefoil.*—Also, roots of birdsfoot trefoil (*Lotus corniculatus*) were examined for the condition of the nodules. The plants had grown in the open field and were blooming at the time of examination. Previously the soil had become so dry that nearly all species of plants growing on it wilted badly during the hotter part of the day. About 2 inches of rain came 7 days before the roots were examined, on August 6.

Nodules on these roots were of various ages physiologically. Some were white and appeared to be newly formed and were turgid; others appeared waterlogged and somewhat soft; while still others were almost black and on handling with forceps would collapse easily, exuding a pasty substance. On one plant 55 nodules were found in this advanced state of decomposition. Sections of both types of nodules were made and examined microscopically before and after staining. It was evident that much starch was present in the sound nodules, only a little in those that appeared waterlogged, and scarcely any or none at all in those that were soft and turning brown or black.

*Soybean.*—Also on August 6, plants of soybean (*Glycine max*) 18 to 24 inches high, just beginning to bloom, were taken from the soil for nodular study. The soil up to one week before had gradually become drier. At this time about 2 inches of rain came. Only a few nodules were seen with diameters less than  $\frac{1}{8}$  inch. Many measured more than  $\frac{5}{16}$ . A total of 108 were examined and of these it was observed that 5 were showing signs of disintegration. They were brown and two of these were distinctly soft. The content of nitrogen in these 108 nodules was 4.53% of the dry weight.

#### CONTENT OF NITROGEN IN YOUNG AND IN SENILE NODULES OF CERTAIN SPECIES OF LEGUMES GROWN IN THE FIELD

If clipping of clover causes a shedding of the nodules through disintegration, it would appear that some of the nitrogen in the nodule might be released while the nodules are still in contact with the plant. This should be suspected if the report (9) in the literature is true that young growing nodules may excrete more nitrogen when growing in association with a nonlegume than the legume itself contains at maturity. The early autolysis in the nodule after clipping might promote such an excretion. From analyses reported in the literature and from the summary of the percentage of nitrogen in nodules of various ages as given by Fred, Baldwin, and McCoy (1), together with their conclusions from a review of this subject, it would appear that the young nodules contain a larger percentage of nitrogen than the older ones.

Preliminary analyses of nodules from wild white clover in various stages of development and senility indicated that some variations



may occur. Difficulties were encountered, however, in obtaining sufficient nodules free of portions of the roots and particles of soil so that reliable data could be obtained. Accordingly, nodules in various periods of their life cycle were obtained from individual plants of *Lotus corniculatus* for this purpose. The information obtained from a typical analysis is presented in Table 1.

TABLE 1.—Nitrogen content of nodules from one plant of *Lotus corniculatus*.

Description of nodules	Weight of sample, mg	% N of dry weight
1st size, smallest, white, no netting on surface, no waterlogging.....	27	9.87
2nd size, slightly pink, some netting, no waterlogging.....	34	10.28
3rd size, all pink, much netting, no waterlogging.....	43	10.59
4th size, losing pink color, heavily netted, signs of waterlogging.....	101	10.61
5th size, all brown to black, with much waterlogging.....	65	9.10
6th size, hulls, ready to burst, black, wrinkled.....	36	6.16

The plant of birdsfoot trefoil from which the nodules came was taken from a sandy soil in the open field late in October. The new growth was heavy. The roots were washed carefully and the nodules stripped from them, then they were placed on cheesecloth and washed. After the various groups were made, each group was carefully scrutinized for foreign material that might modify the percentage of nitrogen.

Although the six groups were arbitrary, they appeared logical. Enough of each group was obtained to give at least 3 mg of nitrogen. Controls were provided.

The results showing the smallest percentage of nitrogen as being present in the smallest white nodules, first size, taken before they were large enough to have developed any visible pink color or show the development of any netting on their surface were unexpected. It was equally surprising that the nodules in the third and fourth groups should contain the highest percentage of nitrogen. The gradual increase of nitrogen from 9.87% to 10.61% gives the reverse order for the nitrogen content of young and of old nodules of red clover as recorded by Wozak according to Fred, Baldwin, and McCoy (1). In another test the nitrogen content in the nodules of the first group was 6.82% and in the fourth group 11.82%.

The reason for this order was not understood at first. It was recalled, however, that the statement was made that as the nodules approach maturity they lose their starch. Therefore, sections were made of the nodules from each of the first five groups. The preparations revealed abundant starch in the young nodules of group 1 and little or none at all in the nodules of group 4. It would appear then that the starch increased the nonnitrogenous portion of the nodule and thus reduced the percentage of nitrogen when expressed as the percentage of the dry weight.

A review of the distribution of nitrogen in the roots and in the

nodules of plants has been made by Fred, Baldwin, and McCoy (1). It includes data from 16 papers and gives the percentage of nitrogen in the nodules of 15 species of legumes. Of this number only three possessed a content of nitrogen larger than 10%. Nodules from birdsfoot trefoil containing little or no starch and thus probably physiologically mature possessed at least 1% more nitrogen than the highest of these three.

## DISCUSSION

The life of a nodule on annual legumes is measured by the life span of each species. In the case of such crops as peas and beans the nodules develop and approach maturity along with the upper parts of the plant. When pod formation and seed production begin, it is reported that the tissues of the nodule become necrotic, turn brown, and, subsequently, contain no energy for the bacteria in the nodule. At this time the content of the nodule which possessed a large percentage of nitrogen has moved up to the fruit. After this movement is completed the empty shell of the nodule remains in the soil and decomposes like other parts of the root system.

When wild white clover was clipped after reaching a height of 4 inches, some of the nodules appeared to pass through the same changes as those on the annuals at maturity. Shortly after the clipping, the nodules on the clover appeared waterlogged, became soft, lost their starch, changed color, and eventually disintegrated. The contents of the nodules apparently were not used by the plants as they were in the case of the peas and beans but were liberated in the soil. The clipping simulated maturity. At initiation of new growth the plants drew on the root systems and nodules for carbohydrates and other substances. Such a demand robbed the nodule and its bacterial content of their support. If this withdrawal is sufficiently severe then the nodule may pass through the stages described above and be released to the soil as organic matter.

Since factors other than clipping may cause the plant to shed its nodules, it is difficult to say how extensively this phenomenon occurs in pasture management where the grasses and perennial legumes are moderately or severely defoliated by grazing or stunted by other factors. It would appear, however, if such factors as defoliation or drying out of the soil are repeated often or occasionally throughout the growing season, and a few or many new nodules appear each time, that significant amounts of nitrogen may be released from the decomposing nodules and become available to the associating nonlegume. It is possible that this liberation of nitrogen and other substances may account for much of the benefits accruing to the nonlegume from its association with the legume in many phases of crop production. Such an interpretation is in accord with the suggestion of Lyon and Bizzell (7) that the roots of legumes slough off organic matter containing nitrogen and that the nitrogen is subsequently taken up by the nonlegume. On the other hand, the data offer apparently scarcely any evidence on the question of whether the developing nodule excretes nitrogen during its sojourn on the plant and that this ex-

may occur. Difficulties were encountered, however, in obtaining sufficient nodules free of portions of the roots and particles of soil so that reliable data could be obtained. Accordingly, nodules in various periods of their life cycle were obtained from individual plants of *Lotus corniculatus* for this purpose. The information obtained from a typical analysis is presented in Table 1.

TABLE 1.—*Nitrogen content of nodules from one plant of Lotus corniculatus.*

Description of nodules	Weight of sample, mg	% N of dry weight
1st size, smallest, white, no netting on surface, no waterlogging.....	27	9.87
2nd size, slightly pink, some netting, no waterlogging.....	34	10.28
3rd size, all pink, much netting, no waterlogging.....	43	10.59
4th size, losing pink color, heavily netted, signs of waterlogging.....	101	10.61
5th size, all brown to black, with much waterlogging.....	65	9.10
6th size, hulls, ready to burst, black, wrinkled.....	36	6.16

The plant of birdsfoot trefoil from which the nodules came was taken from a sandy soil in the open field late in October. The new growth was heavy. The roots were washed carefully and the nodules stripped from them, then they were placed on cheesecloth and washed. After the various groups were made, each group was carefully scrutinized for foreign material that might modify the percentage of nitrogen.

Although the six groups were arbitrary, they appeared logical. Enough of each group was obtained to give at least 3 mg of nitrogen. Controls were provided.

The results showing the smallest percentage of nitrogen as being present in the smallest white nodules, first size, taken before they were large enough to have developed any visible pink color or show the development of any netting on their surface were unexpected. It was equally surprising that the nodules in the third and fourth groups should contain the highest percentage of nitrogen. The gradual increase of nitrogen from 9.87% to 10.61% gives the reverse order for the nitrogen content of young and of old nodules of red clover as recorded by Wozak according to Fred, Baldwin, and McCoy (1). In another test the nitrogen content in the nodules of the first group was 6.82% and in the fourth group 11.82%.

The reason for this order was not understood at first. It was recalled, however, that the statement was made that as the nodules approach maturity they lose their starch. Therefore, sections were made of the nodules from each of the first five groups. The preparations revealed abundant starch in the young nodules of group 1 and little or none at all in the nodules of group 4. It would appear then that the starch increased the nonnitrogenous portion of the nodule and thus reduced the percentage of nitrogen when expressed as the percentage of the dry weight.

A review of the distribution of nitrogen in the roots and in the

nodules of plants has been made by Fred, Baldwin, and McCoy (1). It includes data from 16 papers and gives the percentage of nitrogen in the nodules of 15 species of legumes. Of this number only three possessed a content of nitrogen larger than 10%. Nodules from birdsfoot trefoil containing little or no starch and thus probably physiologically mature possessed at least 1% more nitrogen than the highest of these three.

#### DISCUSSION

The life of a nodule on annual legumes is measured by the life span of each species. In the case of such crops as peas and beans the nodules develop and approach maturity along with the upper parts of the plant. When pod formation and seed production begin, it is reported that the tissues of the nodule become necrotic, turn brown, and, subsequently, contain no energy for the bacteria in the nodule. At this time the content of the nodule which possessed a large percentage of nitrogen has moved up to the fruit. After this movement is completed the empty shell of the nodule remains in the soil and decomposes like other parts of the root system.

When wild white clover was clipped after reaching a height of 4 inches, some of the nodules appeared to pass through the same changes as those on the annuals at maturity. Shortly after the clipping, the nodules on the clover appeared waterlogged, became soft, lost their starch, changed color, and eventually disintegrated. The contents of the nodules apparently were not used by the plants as they were in the case of the peas and beans but were liberated in the soil. The clipping simulated maturity. At initiation of new growth the plants drew on the root systems and nodules for carbohydrates and other substances. Such a demand robbed the nodule and its bacterial content of their support. If this withdrawal is sufficiently severe then the nodule may pass through the stages described above and be released to the soil as organic matter.

Since factors other than clipping may cause the plant to shed its nodules, it is difficult to say how extensively this phenomenon occurs in pasture management where the grasses and perennial legumes are moderately or severely defoliated by grazing or stunted by other factors. It would appear, however, if such factors as defoliation or drying out of the soil are repeated often or occasionally throughout the growing season, and a few or many new nodules appear each time, that significant amounts of nitrogen may be released from the decomposing nodules and become available to the associating nonlegume. It is possible that this liberation of nitrogen and other substances may account for much of the benefits accruing to the nonlegume from its association with the legume in many phases of crop production. Such an interpretation is in accord with the suggestion of Lyon and Bizzell (7) that the roots of legumes slough off organic matter containing nitrogen and that the nitrogen is subsequently taken up by the nonlegume. On the other hand, the data offer apparently scarcely any evidence on the question of whether the developing nodule excretes nitrogen during its sojourn on the plant and that this ex-

cretion is the major source of the nitrogen assimilated by a non-legume as promulgated by Virtanen (9).

### SUMMARY AND CONCLUSIONS

Wild white clover was grown under controlled conditions in the greenhouse to a height of about 4 inches and periodically clipped to a height of about  $\frac{1}{2}$  inch. Such a procedure probably simulates the maturing physiological conditions of many legumes and corresponds to the defoliation which such plants receive under conditions of good grazing management. It also imposes conditions on the root system similar to those which influence adversely the continuity of the nodules on the roots. This imposition reflects itself in the liberation of the nitrogen through the shedding and simultaneous decomposition of the nodules. This has provided a possible mechanism by which a nonlegume growing in association with a legume may obtain at least a portion of its nitrogen from the latter. Knowing that the nodules usually contain a high content of nitrogen, particular attention was given to the effect of defoliation on their disappearance and reappearance with new growth.

It was noted that defoliation like shading, drying out of the soil, or maturity of the plant, caused some of the nodules to be shed and subsequently to decay. It was noted also that at the initiation of new growth the starch in the nodules largely disappeared. If this withdrawal were sufficiently severe the nodules became waterlogged and usually disintegrated. This might account for the higher content of nitrogen expressed as percentage of the dry weight in the old nodules as compared to that in the new nodules. The disintegration of the nodules, liberating their high content of nitrogen in the soil, may also account for much of the increase of nitrogen in the nonlegume growing in association with the legume.

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PLOT TECHNIC STUDIES WITH SMALL GRAINS<sup>1</sup>E. E. DOWN<sup>2</sup>

MODERN methods of plant breeding require the testing of a large number of strains in a limited space. This requirement has prompted many studies (2, 6, 7, 8, 9)<sup>3</sup> of plot technic, some of which have dealt with competition between contiguous plots. The studies reported in this paper have to do with the width of plot necessary to off-set competition effects arising between contiguous plots of winter wheat and between contiguous plots of spring barley. This study was conducted under moisture conditions more favorable for plant growth than most similar experiments have been.

## MATERIALS AND METHODS

All plantings were made with a small nursery planter. The rows were planted 1 foot apart, 18 feet long, and trimmed to 16 feet long prior to harvest. There were no alleyways between plots of a given width. There were four different treatments and all four were planted each year, 1932-38. Each treatment involves two contrasting rates of seeding of one variety or two contrasting varieties planted at the same rate of seeding. The contrasting elements of a treatment were planted alternately so that each side of a plot would have the same type of planting next to it (a condition not possible under simple randomization). The end plots of a treatment had appropriate plantings next to them to maintain this requirement. Ten replications were used of each rate of seeding or variety and four plot widths were involved, *viz.*, one, three, five, and seven rows. The number of plots per treatment was 80; 2 (rates of seeding or varieties)  $\times$  10 (replications)  $\times$  4 (widths). The total number of harvested rows for each treatment was 320; 2 (rates of seeding or varieties)  $\times$  10 (replications)  $\times$  16 (1 + 3 + 5 + 7 rows per plot).

Treatment 1. Plots planted alternately to light (1 bu.) and heavy (2½ bu.) rate of seeding of a variety of winter wheat.

Treatment 2. Plots planted alternately to high-yielding (A) and low-yielding (B) varieties of winter wheat. Each variety was sown at 1½ bushels per acre.

Treatment 3. Plots planted alternately to light (1 bu.) and heavy (3 bu.) rates of seeding of spring barley.

Treatment 4. Plots planted alternately to high-yielding (A) and low-yielding (B) varieties of spring barley. Each variety was sown at 2 bushels per acre. In treatment 2 and 4 the varieties were chosen because of contrasting yields.

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 480.

All rows were planted in a north-south direction. Special equipment (1) developed at this station was used in planting, harvesting, and threshing. At harvest, each row of grain of a plot was covered with a 25-pound paper grocery bag to prevent loss from shattering. Duplicate weights of threshed grain were recorded in grams. Weather conditions were so unfavorable for wheat and barley in 1935 and 1937 that the plots were not harvested.

### NOMENCLATURE

The following designations are used to simplify tabular headings and to clarify the discussion. If two or more rows are involved, it is understood that their mean was used.

One-row plot:

1, single row

Three-row plot:

3, center row

3, (o) two outside border rows

3, all three rows

Five-row plot:

5, center row

5, (s) two second border rows

5, three center rows

5, (o) two outside border rows

5, all five rows

Seven-row plot:

7, center row

7, (t) two third border rows

7, three center rows

7, (s) two second border rows

7, five center rows

7, (o) two outside border rows

7, all seven rows

It is seen from the above designations that the outside border rows (o) are rows 1 and 3 of the three-row plot, 1 and 5 of the five-row plot, and 1 and 7 of the seven-row plot; the second border rows (s) are rows 2 and 4 of the five-row plot and 2 and 6 of the seven-row plot; the third border rows (t) are rows 3 and 5 of the seven-row plot.

The high-yielding variety is designated as variety (A) and the low-variety as variety (B) in treatments 2 and 4.

### RESULTS

In Table 1 are presented the 5-year averages of the yields per row for entire plot and designated portions of the plot for each of the four treatments. A depressing effect on the border rows of a low-yielding plot by having next to them high-yielding plots is clearly shown by the data in this table. For example, under treatment 1, the two outside border rows, 3, (o) in the light rate of seeding average 280.5 grams which is less than their corresponding center row, 3, with an average of 307.5 grams. A similar situation exists when the outside



border rows of five- and seven-row plot are compared with the remaining central portions. These differences are statistically significant. While the yields of the border rows of the low-yielding plots are depressed, the yields of the border rows of the adjoining high-yielding plots are raised. The resultant effect of comparing the yields from whole plots when there is considerable competition is then obvious. The yields from the low-yielding plots are lower than they should be and from the high-yielding plots higher than they should be.

The distance into a plot that competition modified yields is well shown by the relative yield percentages (last column of Table 1). It

TABLE 1.—*The 5-year average yields for the several portions of the one-, three-, five- and seven-row plots, the differences between the yields of the two plantings within a treatment, and the ratios of these yields.*

Treatment 1, Heavy and Light Rates of Seeding Wheat				
Plot portion	Heavy (2½ bu.), grams	Light (1 bu.), grams	Difference, grams	Comparative yields, light/heavy, %
11.....	365.7	253.0	112.7	69.18
31.....	347.6	307.5	40.1	88.46
31(o).....	372.3	280.5	91.8	75.34
31.....	364.1	289.5	74.6	79.51
51.....	320.5	306.6	13.9	95.66
51(s).....	325.6	304.0	21.6	93.37
51.....	323.9	304.9	19.0	94.13
51(o).....	356.2	277.0	79.2	77.76
51.....	336.8	293.8	43.0	87.23
71.....	328.1	315.9	12.2	96.28
71(t).....	326.4	314.5	11.9	96.35
71.....	326.9	314.9	12.0	96.33
71(s).....	325.9	314.7	11.2	96.56
71.....	326.5	314.8	11.7	96.42
71(o).....	360.3	286.8	73.5	79.60
71.....	336.2	306.8	29.4	91.25

Treatment 2, High (A)- and Low (B)-yielding Varieties of Wheat

Plot portion	High (A), grams	Low (B), grams	Difference, grams	Comparative yields, B/A, %
11.....	401.6	223.9	177.7	55.75
31.....	359.1	266.5	92.6	74.21
31(o).....	364.5	243.0	121.5	66.67
31.....	362.7	250.9	111.8	69.18
51.....	355.2	295.9	59.3	83.30
51(s).....	360.3	290.6	69.7	80.66
51.....	358.6	292.4	66.2	81.54
51(o).....	383.8	258.7	125.1	67.40
51.....	368.7	278.9	89.8	75.64
71.....	350.8	313.1	37.7	89.25
71(t).....	350.2	306.4	43.8	87.49
71.....	350.4	308.6	41.8	88.07
71(s).....	353.9	310.9	43.0	87.85
71.....	351.8	309.5	42.3	87.98
71(o).....	379.1	269.4	109.7	71.06
71.....	359.6	298.1	61.5	82.90

TABLE 1.—*Concluded.*

Treatment 3, Heavy and Light Rates of Seeding Barley				
Plot portion	Heavy (3 bu.), grams	Light (1 bu.), grams	Difference, grams	Comparative yields, light/heavy, %
1 <sub>1</sub> .....	364.4	173.5	190.9	47.61
3 <sub>1</sub> .....	286.2	224.2	62.0	78.34
3 <sub>2</sub> (o).....	314.5	196.5	118.0	62.48
3 <sub>3</sub> .....	305.1	205.8	99.3	67.45
5 <sub>1</sub> .....	279.8	214.6	65.2	76.70
5 <sub>2</sub> (s).....	283.1	220.0	63.1	77.71
5 <sub>3</sub> .....	282.0	218.2	63.8	77.37
5 <sub>2</sub> (o).....	311.2	196.7	114.5	63.21
5 <sub>5</sub> .....	293.7	209.6	84.1	71.36
7 <sub>1</sub> .....	297.4	244.1	53.3	82.08
7 <sub>2</sub> (t).....	294.1	249.9	49.2	83.27
7 <sub>4</sub> .....	295.2	244.6	50.6	82.86
7 <sub>2</sub> (s).....	295.0	237.1	57.9	80.37
7 <sub>5</sub> .....	295.1	241.6	53.5	81.87
7 <sub>2</sub> (o).....	320.0	219.6	100.4	68.62
7 <sub>7</sub> .....	302.2	235.3	66.9	77.86

Treatment 4, High (A)- and Low (B)-yielding Varieties of Barley

Plot portion	High (A), grams	Low (B), grams	Difference, grams	Comparative yield, B/A, %
1 <sub>1</sub> .....	304.4	249.6	54.8	82.00
3 <sub>1</sub> .....	298.8	258.5	40.3	86.51
3 <sub>2</sub> (o).....	302.6	258.6	44.0	85.46
3 <sub>3</sub> .....	301.4	258.6	42.8	85.80
5 <sub>1</sub> .....	317.0	285.0	32.0	89.90
5 <sub>2</sub> (s).....	316.5	269.1	47.4	85.02
5 <sub>3</sub> .....	316.7	274.4	42.3	86.64
5 <sub>2</sub> (o).....	325.2	271.6	53.6	83.52
5 <sub>5</sub> .....	320.1	273.3	46.8	85.38
7 <sub>1</sub> .....	319.8	289.9	29.9	90.65
7 <sub>2</sub> (t).....	317.7	299.1	18.6	94.14
7 <sub>3</sub> .....	318.4	296.1	22.3	93.00
7 <sub>2</sub> (s).....	311.2	294.1	17.1	94.50
7 <sub>5</sub> .....	315.5	295.3	20.2	93.60
7 <sub>2</sub> (o).....	316.6	287.0	29.6	90.65
7 <sub>7</sub> .....	315.9	292.9	23.0	92.72

is observed that when the border rows are not included the relative yields come to a maximum. In all four treatments this maximum is found in the seven-row plots. The relative yields of the outside border rows, 7<sub>2</sub> (o), is in all cases lower and in the first three treatments the difference was statistically significant. The same is also true of the five-row and the three-row plots. The relative yields of the second border rows, 7<sub>2</sub> (s), though less than the remaining central portion of the plot in the first three treatments are not significantly less. The corresponding data from the five-row plots give the same results.

That competition did not extend with statistically significant effect beyond the outside border rows is further brought out by the data in Table 2. Differences are presented between the yields of the outside border rows, 3<sub>2</sub> (o), 5<sub>2</sub> (o), and 7<sub>2</sub> (o), and of their corresponding next inner row or rows, 3<sub>1</sub>, 5<sub>2</sub> (s), and 7<sub>2</sub> (s); the yields of the second border rows, 5<sub>2</sub> (s) and 7<sub>2</sub> (s), and their next inner row or rows, 5<sub>1</sub> and 7<sub>2</sub> (t); and the yields of the third border rows 7<sub>2</sub> (t) and their next inner rows, 7<sub>1</sub>. Of the 18 differences involving the outside border rows and their next inner rows in treatments 1, 2, and 3, all but 1 were statistically significant. Of the six similar differences in treatment 4 involving varieties of barley, none was statistically significant. It would appear that even though these two varieties were unlike in yielding ability that there were practically no competitive effect. They head and ripen some 7 to 10 days apart which may account for this situation. Of the 16 differences between the second border rows and their next inner rows, only 1 was at all statistically significant. One of the eight differences between the third border rows and their next inner rows was statistically significant. In Table 1, treatment 4, variety B, the average yield of the center row of the five-row plot, 5<sub>1</sub>, was slightly high and that of the center row of the seven row plot, 7<sub>1</sub>, was slightly low as shown by the comparative yields of B/A. An examination of the original data found these variations to be due to 1 year's results. This, together with the fact no other statistically measurable differences are shown with variety B, indicates that these two measurable differences must be due to some cause other than competition between varieties.

TABLE 2.—Showing the differences between the 5-year average yields of the outside border rows and their next inner rows, of the second border rows and their next inner rows, and of the third border rows and their next inner rows.

Treatment	Winter wheat				Spring barley			
	1		2		3		4	
	Rate of seeding		Variety		Rate of seeding		Variety	
	Heavy, grams	Light, grams	A, grams	B, grams	Heavy, grams	Light, grams	A, grams	B, grams
3 <sub>2</sub> (o)-3 <sub>1</sub>	24.7	-27.0*	5.4	-23.5	27.7	-27.7	3.8	-0.1
5 <sub>2</sub> (o)-5 <sub>2</sub> (s)	30.6	-27.0	23.5	-31.9	28.1	-23.3	8.7	2.5
7 <sub>2</sub> (o)-7 <sub>2</sub> (s)	34.4	-27.9	25.2	-41.5	25.0	-17.5	5.4	-7.1
5 <sub>2</sub> (s)-5 <sub>1</sub>	4.1	-2.6	5.1	-5.3	3.3	5.4	-0.5	-15.9
7 <sub>2</sub> (s)-7 <sub>2</sub> (t)	-0.5	-0.2	3.7	4.5	0.9	-7.8	-6.5	-5.0
7 <sub>2</sub> (t)-7 <sub>1</sub>	-1.7	-1.4	-0.6	-6.7	-3.3	-0.8	-2.1	9.2

\*Negative signs indicate that the yield of the inner row or rows was greater than the next outer row. A difference has to be greater than 9.0 to exceed the 5% point and greater than 16.1 to exceed the 1% point.

These data indicate that when competition effects are present to the extent that they were in these plots with different rates of seeding

or different varieties these effects materially distorted the yields of the outside border rows ( $3_2$  (o),  $5_2$  (o), and  $7_2$  (o)), but that this distortion did not extend to the second border rows ( $5_2$  (s) and  $7_2$  (s)).

The correlation coefficient is sometimes used to show that the mean yield of the central portion of a plot and of the entire plot are so highly correlated that it makes little difference which result is used for comparison purposes. The data available from this experiment indicate that several precautions must be taken in order to place a proper evaluation on the information given by the correlation coefficient. First, the data correlated should consist of the original plot yields rather than yearly averages. The correlation coefficient between the border rows of the three-row plots and their center row when analyzed by covariance (4) on the annual means was 0.948 (footnote Table 3) after the covariance due to treatments and years had been eliminated. This same relationship when determined on the basis of individual plots within a treatment with the covariance due to years eliminated gave coefficients (Table 3) ranging from 0.057 for the heavy rate of seeding for wheat up to 0.705 for variety A of barley with an average coefficient of but 0.459. This latter value is much less than that of 0.948 obtained on the basis of annual means. The difference is statistically significant.

TABLE 3.—*The coefficients of correlation between the outside border rows and the remaining portion of the plot.\**

	Winter wheat				Spring barley				Com- bined†
Treat- ment	1		2		3		4		
Correlation between	Rates of seeding		Varieties		Rates of seeding		Varieties		
	Heavy	Light	A	B	Heavy	Light	A	B	
3 <sub>2</sub> (o):3 <sub>1</sub> ..	0.057	0.293	0.408	0.422	0.667	0.213	0.705	0.288	0.459
5 <sub>2</sub> (o):5 <sub>1</sub> ..	0.587	0.706	0.498	0.513	0.561	0.640	0.592	0.621	0.593
7 <sub>2</sub> (o):7 <sub>1</sub> ..	0.726	0.847	0.620	0.483	0.909	0.778	0.842	0.776	0.773

\*The covariance effect of years was eliminated. The number of variates is 50 as there were 10 replications each of 5 years.

†A coefficient must be greater than 0.287 to exceed the 5% level of significance and greater than 0.372 to exceed the 1% level. The combined values were obtained by Love's (3) method. The three coefficients comparable to the combined values but obtained by using the annual mean yields for all treatments and eliminating the covariance due to years and treatments are 0.948, 0.969, and 0.974 for the three-, five-, and seven-row plots, respectively, and all three exceed the 1% level of significance.

Second, the correlation coefficient measures the tendency of correlated items to act in the same manner regardless of whether the differences between pairs of values are statistically significant or not. For example, in Table 2, it is observed that major competition effects did not show up in treatment 4 as they did in the other three treatments, yet the correlation coefficient between the outside border rows of the three-row plot and their corresponding center rows of variety A of treatment 4 was 0.705, while that of the 3-bushel rate of treatment 3, with highly statistical significant differences between outside border rows,  $3_2$  (o), and center rows,  $3_1$ , was 0.667.

The differences between these two correlation coefficients is non-significant. Again, the correlation coefficient for variety B of treatment 4 was only 0.288, while that of the 1-bushel rate of treatment 1 was 0.293; another negligible difference in spite of the fact that competition effects have been shown to exist in treatment 1 but not in treatment 4 (Tables 1 and 2). These correlation coefficients merely indicate that in the case of the low coefficients of correlation the difference between paired border rows and the center row was highly variable, while in the case of the high coefficient of correlation this difference was rather constant regardless of whether the statistically significant differences (treatment 3) or nonsignificant differences (treatment 4) were involved.

To see whether these relationships were peculiar to these data, the writer obtained a part of Stadler's (5) original data and calculated the same relationships as here determined. A similar situation was found to exist.

For the reasons mentioned above, it is concluded that the coefficient of correlation is inadequate to determine whether border rows should or should not be included in the plot yield.

#### MINIMUM WIDTH OF PLOT

The question of how wide a plot to use is answered, at least in part, by the differences of the two varieties within a treatment. For example, in treatment 1, do the differences between the two rates of seeding tend to remain constant as the plot is made wider? Such differences are given in Table 1 but have been brought together in Table 4 for sake of clearness. The differences between the 5-year average yields of the heavy and light rates of seeding wheat (treat-

TABLE 4.—The differences between the 5-year average yields of the two rates of seeding or of the two varieties of a treatment with the standard errors of a difference between two entries.

Treatment	Winter wheat		Spring barley	
	1	2	3	4
Portion of plot used	Rates of seed-ing, grams	Varieties, grams	Rates of seed-ing, grams	Varieties, grams
1 <sub>1</sub> .....	112.7	177.7	190.9	54.8
3 <sub>1</sub> .....	40.1	92.6	62.0	40.3
5 <sub>1</sub> .....	13.9	59.3	65.2	32.0
5 <sub>3</sub> .....	19.0	66.2	63.8	42.3
7 <sub>1</sub> .....	12.2	37.7	53.3	29.9
7 <sub>3</sub> .....	12.0	41.8	50.6	22.3
7 <sub>5</sub> .....	12.9	42.3	53.5	20.2
S.E. of a difference between two entries.....	10.0*	9.7	11.0	12.3

\*A difference of approximately twice the values given for S.E. difference are needed to be statistically significant at the 5% point within their respective treatments.

ment 1) were 112.7 grams for the one-row plot and 40.1 grams for the center row of the three-row plot. The effect of competition in depressing the yield of the light rate of seeding and in elevating the yield of the heavy rate of seeding, as shown by the one-row plots, have been partially eliminated by using only the center row of the three-row plot. Similar results were obtained in the other three treatments, indicating that yields from one-row plots are so subjected to competition influences that they should not be used.

When the width of plot was increased from three rows up to five, two width of plots were possible without using the outside border rows, namely, the center row only ( $s_1$ ) or the three center rows ( $s_3$ ). In three of the four treatments the differences between single center rows ( $s_1$ ) were less than the corresponding differences between the three center rows ( $s_3$ ), but in no case did the single center row ( $s_1$ ) give results significantly different from those based on the three center rows ( $s_3$ ). In the two treatments involving winter wheat the drop from 40.1 to 19.0 grams in treatment 1 and from 92.6 to 66.2 grams in treatment 2 was a statistically significant drop and indicated that five-row plots gave a better estimation of the true yields than did three-row plots. Such was not the case in the two treatments involving barley. Here there was no advantage in increasing the plot width beyond three rows.

When the width of the plot is increased up to seven rows, three differences are possible without involving the border rows ( $7_1$ ,  $7_3$ , and  $7_5$ ). Within each of the four treatments these differences are very similar. The data from treatment 1 indicate that there is no need for increasing the plot width from five to seven rows for winter wheat. The data from treatment 2 indicate that a seven-row plot should be used. Data from treatments 3 and 4 are consistent in indicating no need for increasing the width of plot beyond the three rows for spring barley.

#### NUMBER OF REPLICATIONS

In the previous section it has been pointed out that for winter wheat the minimum width of plot to be used is five rows and that for spring barley a three-row plot is sufficient. Yields from these two types of plots were used in the determination of the minimum number of replications needed to reduce the annual standard error of the mean to 5% of the mean. The formula used was

$$\text{S.E. mean} = \frac{\text{S.D. sample}}{\sqrt{n}}$$

in which the standard deviation of the sample was expressed as a percentage of the mean. The values of  $n$  calculated for each of the 5 years and for each of the four treatments are given in Table 5. In the case of decimals the values of  $n$  were rounded to the next higher whole number. In general, the number of replications needed for winter wheat was less than the number required for spring barley. The average  $n$  for the 5 years and the two treatments of winter wheat was 3, while the average  $n$  for the spring barley determinations was 7. To determine whether the larger number of replications for barley

was due to the use of data from three-row plots, the values for  $n$  were also calculated for the five-row plots of barley. Although the actual magnitude of the theoretical number was slightly less, the rounded value of  $n$  for the five-row plots was equal to the rounded average number of replications based on three-row plots.

TABLE 5.—*The number of replications needed to reduce the standard error of the mean to 5% of the mean for each of the 5 years for each treatment with 10 replications planted each year.*

Treatment	Winter wheat				Spring barley			
	1		2		3		4	
	Rates of seeding		Varieties		Rates of seeding		Varieties	
	Heavy	Light	A	B	Heavy	Light	A	B
1932.....	2	5	5	2	6	15	13	10
1933.....	3	4	2	2	5	5	9	8
1934.....	2	3	2	2	2	3	7	4
1936.....	2	3	2	2	3	2	4	13
1938.....	3	4	2	1	8	4	3	9
Av.....	3	4	3	2	5	6	7	9
Grand Av.....	3				7			

### SUMMARY

Data collected from winter wheat and spring barley nursery plots involving conditions of severe competition during a 5-year period were analyzed. The data indicate that under the conditions of this experiment the influence of competition did not extend beyond the outside border rows of the plot to a statistically significant extent; the inclusion of the outside border rows of a three- or five-row plot will so distort the results as to give unreliable comparisons; the minimum width of plot to use is a five-row plot for winter wheat and a three-row plot for spring barley, discarding the outside border rows in each case; the average minimum number of replications needed to reduce the annual standard error of the mean to 5% of the mean is three with winter wheat and seven with spring barley; the coefficient of correlation is an inadequate method of determining whether border rows should or should not be used.

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THE EFFECT OF DEW ON THE CURING OF HAY<sup>1</sup>H. B. HARTWIG<sup>2</sup>

IN times when it is difficult or impossible for the farmer to hire seasonal labor, it seems advisable to question the profitability of those practices involved in the harvesting of hay which seem to be merely traditional.

Several years ago, at the writer's request, many of the county agents of New York State designated the best hay-maker in their respective counties. To these "hay-minded" farmers a questionnaire was sent. One of the questions asked concerned the advisability of cutting hay while wet with dew. Thirty-four questionnaires were returned. Twenty-four of the 34 best hay-makers always waited until the dew was off before cutting.

In 1940, the writer solicited, by correspondence, the opinions of 12 agronomists in 12 northeastern states concerning the importance of dew in hay making. Seven of the 12 were of the opinion that hay should not be cut until the dew had dried; a belief also held by the writer. Two were of the opinion that various circumstances should affect the decision as to whether hay should be cut with the dew on or with the dew off. Three seemed to believe that the dew made relatively little difference or that other considerations, mainly labor, were more important. Since the literature contains only opinions unsupported by experimental data, an investigation was undertaken to determine the facts.

## PROCEDURE

The work extended over a period of two years and dealt with the following: First cutting alfalfa (*Medicago sativa*) yielding over 2 tons in 1940 and 1½ tons in 1941; second cutting alfalfa yielding a little less than 1 ton each year; timothy (*Phleum pratense*) stands yielding 1 ton and 1½ tons in 1940 and ¾ ton in 1941; and a mixture of orchard grass (*Dactylis glomerata*) and bluegrass (*Poa compressa* and *P. pratensis*) yielding about 1 ton each year. No study was made which included red clover (*Trifolium pratense*) or soybeans (*Soja max*).

The usual procedure was to cut one swath at about 8 a.m. on mornings when there was a heavy dew, then a parallel swath was cut after the dew had dried. The later time was most frequently between 11 and 12 o'clock, although on some occasions it was as early as 10 a.m. or as late as 1 p.m.

The hay remained in the swath until approximately 5 p.m. on the day of cutting, at which time samples ranging from 3 to 5 pounds were taken. It was necessary in 1940, because of weather conditions, to leave five pairs of first cutting alfalfa swaths and an equal number of the second cutting in the field an extra 24 hours or more before sampling. In most instances, both the "dew on" and "dew off" swaths were sampled in quadruplicate, although all of the 1940 first cuttings were taken only in duplicate. Each sample included at least three complete vertical sections for the entire width of the swath, taken in three different places.

<sup>1</sup>Contribution from the Department of Agronomy, Cornell University, Ithaca, N. Y. Received for publication February 2, 1942.

<sup>2</sup>Professor of Field Crops. The writer acknowledges his indebtedness to Dr. Richard Bradford, Dr. H. O. Buckman, and D. L. Van Horn for helpful suggestions and for critically examining the manuscript.

In all cases, it was assumed that the end of the usual swath curing period had been reached or nearly reached when the moisture was measured. The samples were taken in the late afternoon because it was believed that the windrowing which was to follow introduced another variable.

All samples, except those taken at Mount Pleasant (8 miles southeast of Ithaca), were weighed to 0.01 pound within 5 to 10 minutes of collecting and then dried in an oven. Although the heating at approximately 170° F for 15 hours did not reduce the moisture below 2%, it did, as shown by previously tested samples, bring the moisture to a constant level.

The year 1940 was regarded generally in New York as a year in which it was difficult to cure hay both because of the amount of rainfall and because of its distribution. On the other hand, 1941 was drier and rainfall distribution favored hay curing despite heavy dews.

In order to facilitate the discussion, the results are presented here in tabular form. Besides the percentage of dry matter, such other pertinent data as will be needed in their evaluation are tabulated.

### DISCUSSION

Table 1 shows that in 1940 there was a higher percentage of dry matter (or a lower percentage of moisture) in first cutting alfalfa mowed with the dew on it than in that cut with the dew off when both were sampled at 5 p.m. of the day of cutting. This result occurred in six of the eight paired comparisons. Differences were small.

In the case of the 1940 second cutting of alfalfa, the hay cut with the dew on was a little drier at 5 p.m. than that mowed with the dew off in six of nine pairs.

The table reveals that during 1941, in seven cases in seven, first cutting alfalfa mowed with the dew on was distinctly and consistently drier at 5 p.m. than that cut with the dew off. Second cutting differences were a little smaller but showed the same tendency in five of seven comparisons.

Thus over two seasons, a wet and a dry one, in 24 cases first and second cutting alfalfa cut with the dew on was drier in the late afternoon of the day of cutting than that mowed after waiting until the dew had dried. The reverse was true only seven times.

Those paired alfalfa comparisons which remained in the swath an extra 24 hours or more in 1940 differed from the ones oven-dried at the end of the first day. Three of the five cuttings and one of the five second cuttings showed differences in favor of cutting with the dew on.

Timothy (at Mount Pleasant), mowed while the dew was on from a stand yielding 1 ton per acre in 1940, was a little drier in late afternoon in five cases in five than that mowed after dew had dried. The 1941 hay crop at Mount Pleasant contained a sprinkling of volunteer hop clover (*Trifolium agrarium*) but was lighter and weedier than in 1940. It yielded only  $\frac{3}{4}$  ton per acre. Cutting was unavoidably delayed until mid-July. Only two comparisons were made. In both of these cases, the "dew-off" sample was the drier one at 5 p.m.

Since the Mount Pleasant timothy represented stands thinner than average, two paired comparisons were made in a heavier stand ( $1\frac{1}{2}$

TABLE 1.—A two-year comparison of dry matter in various hay crops toward end of swath curing period when cutting occurred before and after dew was off.

Crop	Approximate yield, tons per acre	Mean % dry matter at time measured*		Mean difference	Odds†	Number of comparisons	No. individual pairs with differences in same direction as mean
		Cut with dew on	Cut with dew off				
1940 Results							
Alfalfa:							
1st cutting.....	2+	36.96	35.28	1.68			
1st cutting†.....	2+	45.44	44.48	0.96	6.5:1	8	6
2nd cutting.....	1-	44.99	43.25	1.74	2.5:1	5	3
2nd cutting†.....	1-	62.41	63.32	0.91	8.0:1	9	6
Timothy:					1:1	5	4
Mt. Pleasant.....	1-	63.17	61.02	2.15	18.5:1	5	5
Caldwell Field.....	1½	60.39	53.30	7.09	3:1	2	2
Orchard grass & bluegrasses.....	1	60.20	60.39	0.19	<1:1	3	1
1941 Results							
Alfalfa:							
1st cutting.....	1½	52.15	48.81	3.34	12.4:1	7	7
2nd cutting.....	1-	49.09	46.51	2.58	11.5:1	7	5
Timothy.....							
Mt. Pleasant, over-ripe, weedy.....	¾	76.62	79.25	2.63	1.5:1	2	2
Orchard grass & bluegrasses.....	1	63.42	62.74	0.68	1:1	4	2

\*Basis oven-dry weights. All samples taken at 5 p. m. ±15 minutes; 3- to 5-lb. quadruplicate samples weighed to 1/100 lb.

†Student's method.

‡Samples taken one day or more after cutting; all others taken on day of cutting.

\*Basis oven-dry weights. All samples taken at 5 p. m. ±15 minutes; 3- to 5-lb. quadruplicate samples weighed to 1/100 lb.

†Student's t method.

‡Samples taken one day or more after cutting; all others taken same day cut.

tons per acre) of timothy on Caldwell Field (Ithaca) in 1940. Fairly large differences resulted. These, in both cases, indicated an advantage for cutting while the dew was on.

Thus, in seven of nine timothy comparisons, hay cut while wet with dew was drier in the late afternoon than that mowed after the dew had evaporated.

The orchard grass-bluegrass mixture used in this study is, of course, not commonly grown for hay, but the great number of basal leaves suggested the possibility of curing difficulties not encountered in timothy or even in alfalfa. The hay dried unevenly in the swath and, consequently, sample replications did not agree very closely. The results summarized in the table indicate that in only four cases in seven was the "dew-on" grass drier at 5 p.m. than the "dew-off".

As an outgrowth of the main project, three comparisons were also made in 1940 of first cutting alfalfa hay cut late during the previous afternoon with that cut (with the dew on) in the usual way at 8 a.m. on the day of sampling. The results (all in the same direction) are not included in the table but are in agreement with the popular idea that there is advantage in cutting hay in the late afternoon. The mean difference, while only a little over 4%, suggests occasional possibility of satisfactorily storing hay a day sooner than might be possible if cutting was delayed until morning.

#### CONCLUSIONS

During the two seasons (1940 and 1941), 47 paired comparisons were made. In 35 of the 47, the hay was drier at 5 p.m. when cut at 8 a.m. while wet with dew than when mowed later after the dew had dried. In most cases, the differences were not large. However, the results reported here suggest that New York farmers producing alfalfa, timothy, and mixed grass hay under the conditions described would not be justified in delaying the use of labor and machinery until the dew has dried from the standing crop.

BREEDING CORN HYBRIDS FOR SMUT RESISTANCE<sup>1</sup>G. H. STRINGFIELD AND D. H. BOWMAN<sup>2</sup>

CORN smut, *Ustilago zeae* (Beckm.) Unger, is one of the major corn diseases. It cannot be controlled either by seed treatment or by cultural methods. No significant advance was made in the control of corn smut until first-generation hybrids combining homozygous or nearly homozygous inbred lines came into use. Data obtained in Ohio and reported here show, first, that most corn hybrids are less susceptible to smut than are open-pollinated varieties and, second, that several productive hybrids now being grown are resistant to smut, as well as to lodging. The term "resistance" as used in this paper refers to an appreciably lower degree of susceptibility than is characteristic of commonly grown corn.

## REVIEW OF LITERATURE

It is a common observation among corn breeders that inbred lines differ markedly with respect to the occurrence of smut. When such differences appear consistently, they are interpreted as being expressions of differential host susceptibility. In the U. S. Dept. of Agriculture germ plasm survey (10),<sup>3</sup> 391 outstanding inbred corn lines are listed, of which 277, or 71%, are given "smut-resistance" ratings. Differential ratings for at least part of the lines are given for 18 of the 30 corn breeding projects listed.

Jones (11) reported differential and heritable strain reaction to smut in 1918. Hayes, *et al.* (5), Immer and Christensen (8), Garber and Quisenberry (4), Immer (7), Hoover (6), and other investigators have thoroughly corroborated Jones's conclusions and have added valuable information relative to the inheritance of host reactions. All these authors agree that it should be possible to develop desirable hybrids that are resistant to smut.

Immer (7) found a close agreement between the smut reactions for the same inbred lines grown both in Minnesota and in West Virginia, and Christensen and Johnson (1) found that the relative responses of 5 open-pollinated varieties and 95 selfed lines in general were similar when the strains were inoculated with different collections of smut from Minnesota and 12 other states. Localization of infection on the host was found to be a strain characteristic, irrespective of collections of smut used, and different smut collections did not influence the number and size of galls on infected plants.

The smut pathogene, however, is a variable organism. Melchers (12) pointed out the probability of the existence of physiological differences in 1920. Stakman and Christensen, and their associates (2, 13, 14, 15, 16), reporting in several papers on *Ustilago zeae*, have found numerous strains, heterothallism, and fre-

<sup>1</sup>Contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, and the Agronomy and Botany Departments, Ohio Agricultural Experiment Station, Wooster, Ohio. Received for publication February 4, 1942.

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 493.

quent mutation. These strains differed in pathogenicity, as well as in other characters. Variability in the organism was found also by Eddins (3) and Verplaneke (17).

### MATERIALS AND METHODS

The data for the present study were obtained from the regular corn performance experiments used to measure the value of various corn hybrids and open-pollinated varieties. Replication was either four- or fivefold; strain sequence was at random; and the plot size was either 2 by 10 or 2 by 15 hills in each experiment. Smut counts were taken on 1,392 corn entries in 48 experiments in 13 counties well distributed over Ohio during the years 1933 to 1938, inclusive. An entry is one strain in one experiment.

The smut counts recorded were the number of galls observed externally. These simple counts avoided any damage to the plants incident to removing leaf sheaths and, at the same time, permitted the use of some untrained help in making counts. It was assumed that smut galls too small to be visible without removing leaf sheaths were of questionable importance as indicators of host susceptibility. Immer and Christensen (9) had found that susceptible inbred lines tended to have both more numerous and larger smut galls per plant than resistant lines. Small galls, which often appear early on leaves, were not counted, as they seldom become large enough to interfere seriously with host development.

A plant was recorded as smutted when it had a visible gall any place on the main culm or ear. The smutted tassels were ignored because the tassel galls usually were small, difficult to evaluate, and seemingly relatively unimportant. Evidence obtained after the present studies were nearly completed indicated that in future studies attention should be given to smutted tassels. However, the point did not seem to be of sufficient importance to warrant changing after a considerable portion of the data had been collected. It was assumed that plants not developing sufficient gall tissue to interfere measurably with normal host development should not be classed as smutted, and that resistance might be expressed, not only in the absence of initial infection, but also in restricted gall development after infection.

If the galls on resistant strains are fewer but not different in size from those on susceptible strains, the method used should give a correct ranking of the strains. The degrees of difference in susceptibility, however, would be minimized because not all the galls would be counted.

If galls on resistant strains are as numerous but are smaller, the method used should give a correct ranking and should determine more nearly the different degrees of susceptibility because only the larger galls would be important. In this case, it would be necessary to ignore very small galls or real differences would be lost.

It follows that the method used should determine the correct ranking of strains if the galls on resistant strains are both fewer and smaller.

The presence of more than one gall on a plant was disregarded in classifying strains for resistance, in spite of the tendency for susceptible strains to have more galls per plant. In three seasons at Wooster, however, counts of the total number of smut galls, as well as of the percentage of infected plants, were recorded. Comparative coefficients of variation, after the elimination of variance due to strain and replication, are shown in Table 1. It appears that at these low levels of infection, it matters little whether additional galls per plant are included in

the counts or disregarded. These experiments were conducted in fivefold replications in 2- by 10-hill plots in 1935 and 1936 and mostly 2- by 15-hill plots in 1937.

TABLE 1.—*Comparison between counts of smutted plants and counts of total galls in corn tests at Wooster, Ohio, after variation due to strain and to replication was eliminated.*

Year	Number of corn entries	Infection per 100 plants		Entry coefficient of variation	
		Plants	Galls	Plants	Galls
1935.....	70	8.68	10.10	21.20	22.05
1936.....	72	5.28	5.63	24.96	26.61
1937.....	60	5.38	5.97	23.01	24.49

In the field performance experiments from which the data presented here were taken, it was the practice to include at least four locally obtained, adapted, open-pollinated varieties. Some of the certified open-pollinated varieties, such as Medina Pride, Woodburn, Clarage, and Reid, usually were included also in sections where they were adapted. Most of the entries, however, were hybrids. The hybrids were top crosses, single crosses, three-way crosses, or double crosses, and, although most of them were of Ohio origin, many were introduced from outside of the state.

#### DATA AND DISCUSSION

In order to summarize data from many experiments so that a general comparison could be made between hybrids and open-pollinated varieties, the smut infection of each entry was computed as a percentage of the mean infection of comparable open-pollinated varieties in the same experiment. Only experiments containing four or more open-pollinated varieties were used because of the large error in the determination of a single entry. The distribution of smut infection of the open-pollinated varieties as percentages of their own means is shown in Fig. 1. The means averaged 6% infected plants. The large spread results from errors in the readings much more than from inherent varietal differences.

The frequency distribution of smut infection of 1,052 miscellaneous hybrid entries as percentages of the same means as used for Fig. 1, is shown in Fig. 2. Approximately three-fourths of the hybrids showed lower smut percentages than did the open-pollinated varieties in the same experiments. The tendency for the hybrids to appear in the lower smut classes is obvious. With the large number of entries, a statistical study of significance would seem superfluous.

The following 15 inbred lines appeared to have more or less smut resistance: Ohio 02, 10, 17, 15-6, 26, 51, 56, 65, 66, 67, 84, 306A, 601S; C. I. 4-8; and Iowa L<sub>317</sub>. More recent studies have shown lines Ohio 65 and Ohio 67 to be moderately susceptible and Ohio 17 highly susceptible. Each hybrid whose parentage was made up from these "resistant" lines by half or more was tentatively placed in a "resistant" group. This group included each top cross and three-way cross having a "resistant" line as pollinator, and each three-way or

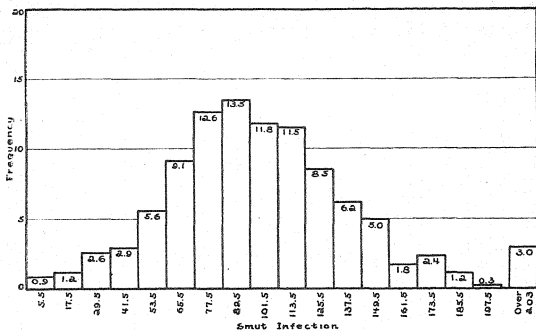


FIG. 1.—Frequency distribution on a percentage basis of smut infection counts in 340 entries of miscellaneous open-pollinated corn strains in 48 experiments. The smut infection of each open-pollinated strain in each experiment was computed as a percentage of the mean smut infection of all open-pollinated strains in the same experiment.

double cross having two "resistant" parents, whether on the same side or on opposite sides, regardless of which other strain or strains were in the hybrid. The distribution of this group, again based upon the mean smut infection of open-pollinated varieties in the respective

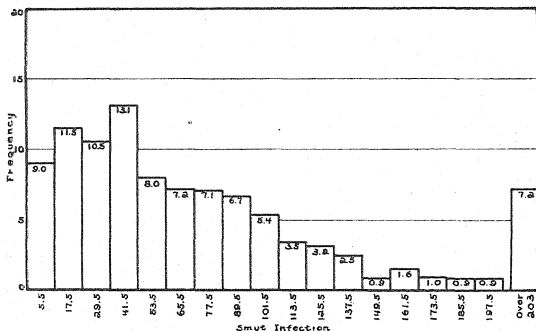


FIG. 2.—Frequency distribution on a percentage basis of smut infection counts in 1,052 entries of miscellaneous corn hybrids in 48 experiments. The smut infection of each hybrid strain was computed as a percentage of the mean smut infection of all open-pollinated strains in the same experiment.



TABLE 2.—*Smul reaction, lodging, and acre yield of four commercial hybrids adapted to northern Ohio compared with five open-pollinated varieties adapted to the same area.\**

	Woodburn	Cook	Medina Pride	Waugh	Clarage	Average (Unweighted)	
						Hybrid	Varieties
Smutted Plants, %							
Hybrid Ohio W17.....	0.8 (20)	0.9 (11)	1.2 (8)	1.2 (5)	1.5 (9)	1.1	5.0
Variety compared.....	3.8	2.7	5.9	6.7	5.7	—	—
Hybrid Ohio K35.....	1.6 (6)	2.0 (3)	3.6 (1)	3.6 (1)	3.6 (2)	2.9	—
Variety compared.....	5.1	8.9	9.0	12.2	13.1	—	9.7
Hybrid Ohio K23.....	1.1 (10)	1.2 (8)	2.5 (7)	2.0 (3)	1.7 (8)	1.7	—
Variety compared.....	3.2	2.6	6.2	6.4	4.9	—	4.9
Hybrid Iowa 939.....	3.1 (17)	2.0 (11)	3.9 (8)	3.1 (4)	3.1 (8)	3.1	—
Variety compared.....	4.3	2.7	5.9	5.4	4.6	—	4.6

	Lodged Plants, %						
	11.1 (39)	7.0 (16)	3.6 (8)	11.3 (7)	10.0 (20)	8.6	24.7
Hybrid Ohio W17.....	24.1	21.3	21.6	33.1	23.4	—	—
Variety compared.....							
Hybrid Ohio K35.....	7.1 (16)	2.8 (7)	0.3 (1)	19.9 (3)	3.6 (4)	6.7	31.2
Variety compared.....	30.5	28.9	27.4	44.6	24.6	—	—
Hybrid Ohio K23.....	4.3 (20)	4.4 (12)	2.6 (13)	3.0 (7)	5.3 (12)	3.9	20.7
Variety compared.....	20.2	21.8	19.5	19.2	23.0	—	—
Hybrid Iowa 939.....	11.5 (35)	7.2 (16)	5.6 (7)	13.6 (7)	10.9 (23)	9.8	24.8
Variety compared.....	23.6	21.3	23.4	33.1	22.7	—	—
Acre Yield, Bu.							
Hybrid Ohio W17.....	77.2 (39)	77.2 (16)	88.2 (8)	83.5 (7)	79.5 (20)	81.1	71.2
Variety compared.....	69.3	70.0	72.1	73.2	71.4	—	—
Hybrid Ohio K35.....	83.2 (16)	90.8 (7)	118.4 (1)	85.1 (3)	97.2 (4)	94.9	77.7
Variety compared.....	76.6	78.5	78.8	75.0	79.8	—	—
Hybrid Ohio K23.....	72.1 (21)	75.4 (12)	82.0 (13)	85.4 (6)	78.1 (12)	78.6	73.1
Variety compared.....	71.8	75.7	69.6	75.0	73.4	—	—
Hybrid Iowa 939.....	78.7 (36)	78.0 (16)	89.3 (9)	85.4 (7)	82.3 (23)	82.7	71.0
Variety compared.....	72.4	70.0	67.2	73.2	72.0	—	—

\*Numbers in parentheses refer to the number of comparisons between a particular hybrid and variety.

experiments, is shown in Fig. 3. The fact that a few of the entries had more smut than the mode for the competing varieties cannot safely be interpreted as meaning anything other than error in some of the individual readings. It is significant that the smut infection in the modal class was less than a third of that of the competing open-pollinated varieties. The inbred lines in the resistant group, except 17, 15-6, 66, 306A, and 601S, are used in certified commercial hybrids in Ohio. One hybrid, Ohio W17, referred to later, involves four of these lines.

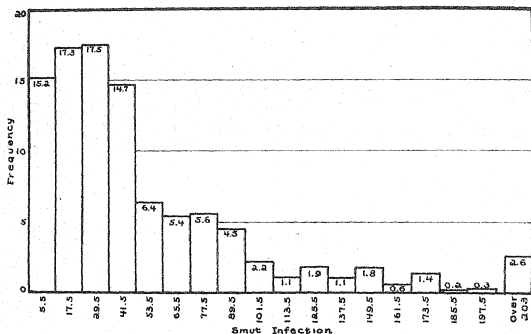


FIG. 3.—Frequency distribution on a percentage basis of smut infection counts in 624 entries of selected hybrids in 41 experiments. Half or more of the parentage of each of these hybrids consisted of inbred strains designated as "smut resistant". As in Fig. 2, the smut counts were computed as percentages of the mean infection of the open-pollinated strains.

Four commercial hybrids, all certified by the Ohio Seed Improvement Association for use in northern Ohio, were compared with five outstanding open-pollinated varieties, Woodburn, Cook, Medina Pride, Waugh, and Clarage (Wooster strain). These five are representative of the better open-pollinated corn varieties formerly grown in the northern half of Ohio.

Since smut resistance is of no immediate economic value unless it is combined with characters of general agronomic value, the available data comparing four commercial hybrids with the five open-pollinated varieties in smut reaction, lodging, and yield are shown in Table 2. At least three of the hybrids as compared with the open-pollinated varieties combine smut resistance with lodging resistance and high grain yields.

Data were collected during four seasons from experiments located in counties bordering Pennsylvania, Indiana, West Virginia, and Kentucky. None of the plantings showed extremely heavy infection. The variable conditions represented by the different experiments and

also Immer's encouraging findings, previously referred to, support the conclusion that these hybrids are resistant to smut in any location in which they are adapted.

The possibility of new forms of the smut pathogene arising or multiplying to nullify the gains that have been made cannot be ignored. However, in view of the statement of Stakman, *et al.* (16) that, "Because of the nature of resistance, however, physiologic specialization now appears relatively unimportant in breeding resistant lines of corn", it is felt that this possibility need cause no immediate concern.

#### SUMMARY AND CONCLUSIONS

Smut counts made in 48 corn performance experiments showed that about three-fourths of the hybrid entries carried less smut infection than did the open-pollinated varieties in the same experiments. Twelve inbred lines appear to be more or less resistant to corn smut. Hybrids in which half or more of the parentage consisted of these resistant inbred lines showed less than a third as much smut as did the open-pollinated varieties.

It is shown that hybrids have been developed that combine a high degree of smut resistance with other desirable agronomic characters. Such hybrids are being grown extensively. The use of these and similar hybrids in other states constitutes the first significant advance in the control of corn smut.

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## A COMPARISON OF BORON DEFICIENCY SYMPTOMS AND POTATO LEAFHOPPER INJURY ON ALFALFA<sup>1</sup>

W. E. COLWELL AND CHARLES LINCOLN<sup>2</sup>

**A** YELLOWING of alfalfa caused by the potato leafhopper, *Empoasca fabae*, was described in 1927 (4)<sup>3</sup> and since that time several papers have dealt with the nature of this injury (1, 2, 3, 6, 7). In describing the yellowing, Granovsky (2) placed emphasis on the striated discoloration of the areas between the lateral veins. He also reported a shortening of the internodes of the new growth, and rosetting of the new shoots.

Boron deficiency symptoms on alfalfa were recognized some 10 years later (5, 8) and in the description of McLarty, Wilcox, and Woodbridge (5), there was emphasized a uniform yellowing and (or) bronzing over the intercostal area of the terminal leaves, a shortening of the internodes, and death of the growing points.

The leafhopper symptoms were described before boron deficiency was known to be a problem, and the symptoms due to a lack of boron were described either in the absence of leafhopper injury or without recognizing it as contributing to the abnormality. Since the existing descriptions are not adequate to differentiate clearly between the two types of injury, a comparison of these symptoms has been made in the greenhouse and under field conditions in New York where both agencies contribute to alfalfa yellowing.

### EXPERIMENTAL

#### GREENHOUSE STUDIES

The general plan of the greenhouse investigation was to infest healthy alfalfa cultures with leafhoppers and to compare these symptoms with those caused by a deficiency of boron under similar conditions.

Forty 2-gallon stone jars containing two or three 18-month-old plants growing in a sub-surface sample of Dunkirk sandy loam which was deficient in boron were used as a source of material.<sup>4</sup> To 20 of these, boric acid to supply 0.046 p.p.m. B (dry weight basis) was added on April 14, 1941. The 20 remaining pots received no added boron. All plants were clipped on April 14. On May 19, six of the boron-treated jars and two of the minus boron jars were each caged and infested with 25 nymphs and 10 adults of the potato leafhopper.

Complete nutrient solution minus boron was supplied to all cultures, and distilled water was used to maintain moisture. They were placed in the regular greenhouse, receiving only natural light. On June 2, final observations were made.

<sup>1</sup>Joint contribution from the Departments of Agronomy and Entomology, Cornell University, Ithaca, N. Y. Received for publication February 6, 1942.

<sup>2</sup>Research Fellow in Soils and Assistant Professor of Entomology, respectively. The senior author wishes to express his appreciation to the American Potash Institute, Inc., for providing the fellowship which made possible his participation in this study. Both authors gratefully acknowledge the Institute's additional assistance in supplementing college funds for the colored plate.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 498.

<sup>4</sup>Acknowledgment is made to Prof. J. K. Wilson who donated these cultures which he had used for other work prior to this time.

Symptoms of boron deficiency and leafhopper injury were each obtained in the absence of the other.

The results of this comparison are presented with those of the field investigations.

#### FIELD INVESTIGATIONS

A further comparison was made in the field during the summer of 1941 at two locations in north central New York, one station designated as Belleville, the other as Lowville. In October, 1940, borax at the rate of 40 pounds per acre was added to an area 20 by 40 feet in a field which, in each case, had been cut for hay only one year. The response to borax was in each case a mild one, but more definite at the first-mentioned station.

During the second crop in 1941, there were set up at each station four aster cloth cages, the dimensions of which were 4 by 4 by 2 feet. Two were set on the borax plot and two in the adjacent untreated area. One of each was infested with 500 leafhoppers, adults and large nymphs. Caging the plants did not noticeably affect growth. Fig. 1 shows the cages in place at the Belleville station.



FIG. 1.—Aster cloth cages at the Belleville station on August 26 just prior to making final observations.

At the Belleville station the natural leafhopper population was low (around 75 per 100 sweeps), but the two uninfested cages were dusted with impregnated pyrethrum to insure their being leafhopper free. At the Lowville station the natural population was still lower, (around 30 per 100 sweeps), and the two uninfested cages were not dusted. Infestations were made at the Belleville station on August 5 and at the Lowville station on July 31. At each location the alfalfa was about 12 inches high and growing luxuriantly. At this time there was no visible response to the added borax.

Semi-weekly observations were made, but the cages were left undisturbed until August 26 when they were removed for the final observations.

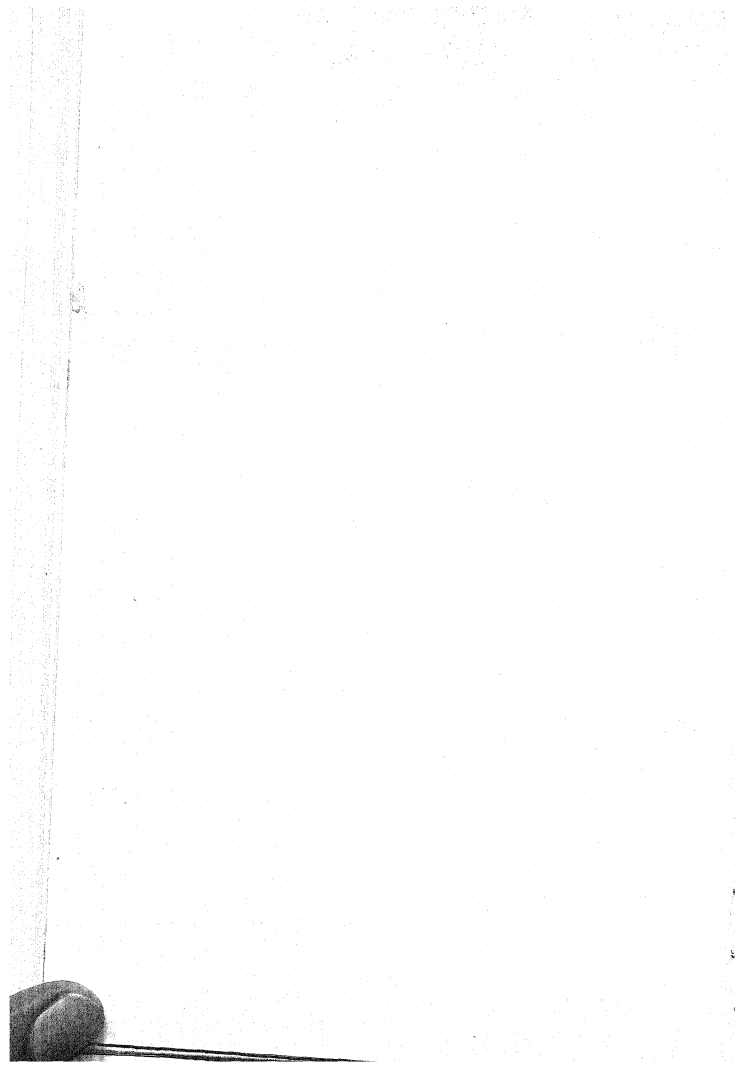
#### RESULTS

As a result of these studies, the following differences between the two types of injury were found to exist. The source of information, greenhouse or field, will be apparent in each case by reference to the various figures.



PLATE I.—A, leafhopper injury in which the reddening is the main type of discoloration. Hopper infested cage, boron plot at the Belleville station. B, severe leafhopper injury, Jefferson County. C, left, leafhopper injury; center, healthy; right, boron deficiency. Greenhouse test. D, boron deficiency showing pronounced rosetting and death of the terminals with little or no yellowing. Greenhouse test. E, severe boron deficiency. Uninfested cage in untreated area at the Belleville station. F, left, an advanced stage of boron deficiency, the lower side of several leaflets are red; right, a moderate stage. Monroe County demonstration.





## TYPE OF DISCOLORATION

*Yellowing.*—The type of yellowing alone is not adequate as a means of differentiation, although there is some tendency for the leafhopper yellowing to be characterized by a streaked appearance in which the veins and mesophyll adjacent to them remain green (Plate I, C). Leafhopper yellowing often takes place at the distal portion of the leaflet as a "V" in which the apex represents the point of feeding,<sup>3</sup> and the sides are bounded by veins extending to the edge of the leaflet. (See certain lower leaflets in Plate I, B.) In other cases the yellowing is more uniform (Plate I, C) and from this standpoint alone cannot be differentiated from the uniform yellowing of boron deficiency (Plate I, E and F). There may be a pronounced boron deficiency with little or no yellowing (Plate I, D).

*Reddening or bronzing.*—Leafhopper reddening occurs either in a "V" as described above (Plate I, A, upper left) or as a uniform discoloration of the entire leaflet (Plate I, B, right). This latter type is difficult to differentiate from boron reddening. In general, the leafhopper reddening tends to have more of a purplish cast, but this is not always true. Boron reddening is believed to represent an alternative symptom, not necessarily a more advanced stage. In New York, yellowing is the more common symptom, but both may occur even on the same leaflet. Many times, though not always, those leaves which are yellow on the upper side may be red underneath. This was true of the severely deficient specimen in Plate I, F.

## DISTRIBUTION OF DISCOLORATION

This is one of the most valuable criteria for distinguishing between the two types of injury. The leaves injured by leafhoppers occur at various heights on a given shoot, whereas the yellowing or reddening caused by boron deficiency is always confined to terminals. Lateral terminals may be affected, and, under these conditions, yellowing is obviously not confined to the top of the plant (Plate I, E).

Although leafhopper yellowing is not confined to terminals but occurs well over the plant, it is not to be confused with the common leaf spot caused principally by *Pseudopeziza medicaginis*. This organism causes small, circular, dark brown spots on the lower leaves, and if sufficiently severe causes them to turn yellow and drop. There is likewise no danger of confusing leafhopper yellowing with that caused by potash deficiency or even by *Phytomonas insidiosa* (bacterial wilt) which, from a distant field view may appear somewhat similar.

## ROSETTING

There is always a shortening of the terminal internode in plants showing boron deficiency (Plate I, C, right, D, E, F). This rosetting may occur even in the absence of pronounced yellowing (Plate I, D, and E, right). Even in severe cases of leafhopper injury, this terminal

<sup>3</sup>The observation of punctures at the points of feeding is not reliable as a method for field diagnosis.

internode is not appreciably affected (Plate I, A, B, and C, left). There may, however, be a general stunting of leafhopper-injured plants.

#### DEATH OF THE TERMINAL

The terminal bud of a boron-deficient alfalfa plant is always found to be abnormal. Dead buds may be found on shoots showing no yellowing, and in this case are sometimes associated with causes other than boron deficiency. However, even in the absence of yellowing, when the dead bud is accompanied by pronounced rosetting, true boron deficiency is represented (Plate I, D).

The terminal growth in those plants severely affected by leafhopper may be normal; in fact, blossoms are often abundant in a field supporting a rather heavy leafhopper population.

During the latter part of the growing season, boron deficiency and leafhopper injury occur in the same fields in several sections in New York. It is not uncommon to find both types of injury on the same plant and this cumulative effect is readily recognized by noting the two types of symptoms.

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## NOTES

### PERSISTENCE OF *RHIZOBIUM JAPONICUM* IN SOIL

IN 1941 an experiment on the relative effects of inoculation on soybean yields on soil of medium and high fertility was laid out on two areas on the Agronomy Farm at Ames. One was a Clarion sandy loam, partly eroded; the other was a Clarion loam that had suffered little or no erosion and had been in alfalfa for some years. Neither was believed to have carried a crop of soybeans during our tenure of this land, nor had beans been planted closely adjacent to either area. Five replicates of each treatment (inoculated and uninoculated) were set up with guard rows between plots.

Forty to 50 plants were dug from each plot early in July and nodules were counted. All plants on the Clarion sandy loam area and 86% of the plants on the Clarion loam area were nodulated and there was no significant difference between inoculated and uninoculated plots in either area. However, between areas there was a major difference. On the high fertility Clarion loam area the nodulated plants bore an average of 3.3 nodules, whereas on the poorer Clarion sandy loam area the number was 19.6 per plant. The lower number of nodules per plant in the former area was no doubt a result of the high nitrogen status, since it has repeatedly been shown that additions of nitrate reduce nodulation. Yields were taken later, and again there was found to be no significant difference as a result of inoculation. The mean yields for the respective areas were 27.4 bushels per acre for the Clarion loam area and 20.9 bushels per acre for the Clarion sandy loam area.

On searching further into the records later it was found that in the season of 1917 immediately before this portion of the farm was taken over for experimental purposes, a crop of beans had been planted on the two ranges concerned. A sufficient number of organisms to produce satisfactory nodulation had therefore been maintained in these soils for a period of 24 years.

There have been other reports of the longevity of *Rhizobium japonicum* in soil in the absence of its symbiont. J. K. Wilson (Cornell Univ. Agr. Exp. Sta. Mem. 162, 1934) in an extensive survey reports one case of persistence for a 25-year period, and Russell and Morrison (Wis. Agr. Exp. Sta. Bul. 319, 1920) one for a 17-year period.

It is clear that under certain conditions this organism, though not usually considered particularly vigorous, can maintain itself for long periods in competition with the ordinary heterotrophic soil population. Such an occurrence, however, cannot be cited as good evidence against the desirable practice of inoculation of soybeans at frequent intervals, in view of the many reports of failure to produce nodulation after comparatively brief periods.

The purpose of this note is to draw attention to the fact that there is yet little information as to those soil factors influencing longevity of rhizobia despite the practical importance of the problem.—A. G. NORMAN, Agronomy Section, Iowa Agricultural Experiment Station, Ames, Iowa.

OBSERVATIONS OF KUDZU, *PUERARIA THUNBERGIANA*  
BENTH, SEEDLINGS<sup>1</sup>

A PRACTICAL method of growing kudzu seedlings has been developed by the Soil Conservation Service nurseries in the southeastern United States. The first trial plantings were made during the spring of 1935. Since that time nearly 84 million kudzu seedlings have been produced in SCS nurseries in the southeastern United States. This number is believed to be approximately 90% of the total production to date in this country.

Kudzu seedlings have several advantages over kudzu "crowns", a common name for plants developed naturally from rooted nodes. The seedlings are produced in one growing season, the yield per acre is higher, and the lifting, storage, transportation, and planting are less difficult.

Securing good stands on non-irrigated land has been the most difficult part of kudzu seedling production. Kudzu seed are small, varying from 30 to 45 thousand per pound, and a large percentage are "hard". The seed skin is relatively thick and brittle. Ordinary mechanical scarification is helpful, but less efficient than desired. Scarification with commercial concentrated sulfuric acid requires extended exposures varying with temperature and lots of seed. An exposure of 1 hour at 60° F was required by one lot of seed. Unless perfect scarification is done the germination of many seed is delayed.

The hypocotyl of the young kudzu seedlings is more limited in power to elongate than that of most field crops. Shallow planting is necessary. A depth of  $\frac{1}{4}$  to  $\frac{1}{2}$  inch has been most successful.

Monthly mean air temperatures of 65° to 75° F have been optimum for field-grown kudzu seedlings. The young seedlings are not frost hardy. Kudzu seedlings are intolerant of water-saturated soils. The roots die below the level of a temporary water table in 1 to 3 days. This presumable intolerance to low oxygen tension may be one of the chief reasons for poor germination of deep-planted seed following hard rains.

Young kudzu seedlings are more tender than most field crops. They are easily injured by soluble commercial fertilizers and by mechanical disturbance. After four or five true leaves have developed, the susceptibility to mechanical injury is much less.

Transplanting kudzu seedlings in the fourth to sixth true leaf stage has been successfully done in one SCS nursery.

Kudzu seedlings have grown to plantable size in 80 days when the seed were placed in the soil during July. A plantable seedling has one or more roots at least  $\frac{3}{8}$  inches in diameter and at least 6 inches long. The root size indicates the amount of food storage, an essential to high survival of transplanted kudzu.

Early planting of kudzu seed encourages relatively heavy vine growth; late planting relatively light vine growth. Presumably long day length is favorable for vine growth and short day length to food storage in the roots.

<sup>1</sup>Acknowledgment is made for assistance in observations of kudzu seedlings to all professional employees of the Nursery Division, SCS, Region 2, and to the Agronomists of the SCS, Regional Office, Spartanburg, S. C.

Kudzu seedlings have been grown successfully on well-drained soils capable of producing 25 bushels or more of corn per acre. The best soils for consistently good growth have been Red Bay fine sandy loam and Red Bay clay loam in Mississippi and Alabama, and similar river-terrace soils from Davidson material in the Piedmont Plateau of Virginia. Each of these soil types is dark red in color, relatively uniform in profile, and has a deep B horizon.

One hundred thousand kudzu seedlings per acre is a practical maximum due to vine competition. Narrow rows and thick planting are necessary for this yield. Such spacing gives a relatively low yield per pound of seed used. Much higher yields per pound of seed but not per acre have been secured from wider (3 foot) rows and a lighter rate of seeding (15 seed per linear foot instead of 25).

Kudzu seedlings have secured sufficient naturally occurring symbiotic nodule-forming bacteria in each place grown. Artificial inoculation of the seed has not increased growth or yield.

Both cessation of growth and partial drying of kudzu seedlings are needed before lifting should begin. During a drought, lifting may be started about the time of the first frost in the fall. During normal or wet periods a wait of 2 to 3 weeks has been necessary to avoid mechanical injury of seedlings just below the ground level.

Clipping the green vines of kudzu seedlings before lifting has seriously injured their cold resistance and keeping quality in Virginia and North Carolina. Clipping during a long fall drought at Americus, Georgia, in 1939 caused no apparent injury. Clipping seedling vines before frost is not recommended under any conditions.

Kudzu seedlings not injured in lifting are easily kept by heeling in on well-drained land.—PAUL TABOR, *Soil Conservation Service, Spartanburg, S. C.*

## AGRONOMIC AFFAIRS

### THE FIRST WESTERN MEETING OF BIOMETRICIANS

UNDER the general title "The potential and Actual Contributions of Statistics to the Solution of Biological Problems", the first meeting of western biologists and mathematicians was held on the Berkeley Campus of the University of California on December 29 to 31, 1941. Of the 24 papers presented, the larger proportion were of direct interest to agronomists and dealt with problems of genetics, plant breeding, and sampling problems in forestry. At these meetings, largely attended by biologists, the discussions provided an opportunity for mathematicians to appreciate some of the biological problems needing solution, and at the same time biologists were made aware of statistical methods available but not widely used.

Those attending were so impressed with the mutual advantages of such meetings that an organizing committee was elected for the purpose of forming a permanent organization which would arrange future meetings and at the same time explore the possibilities of establishing a journal that would bridge the gap between purely biological and mathematical journals. The organizing committee is made up of mathematicians and biologists with the latter predominating. The follow-

ing officers were elected: J. Neyman (Mathematical Statistics), *Chairman*; E. B. Babcock (Genetics), *Vice Chairman*; R. T. Birge (Physics), *Vice Chairman*; E. C. Tolman (Psychology), *Vice Chairman*; E. R. Dempster (Genetics), *Secretary*; and A. Hormay (Forestry), *Secretary*. All are of the University of California at Berkeley.

It is the hope of the organizing committee that the proposed permanent organization will enjoy the approval and cooperation of agronomists. Those interested are requested to communicate with a member of the organizing committee.

#### THE 1941 PROCEEDINGS OF THE SOIL SCIENCE SOCIETY OF AMERICA

UNLESS some unforeseen obstacle intervenes, Volume 6 of the Proceedings of the Soil Science Society of America with the papers presented at the annual meeting of the Society in Washington, D. C., in November 1941 will be ready for distribution the latter part of this month. The book will make 524 pages. Copies may be obtained by addressing requests to Dr. G. G. Pohlman, West Virginia Agricultural Experiment Station, Morgantown, W. Va., Treasurer of the Soil Science Society. The price is \$5.00 post paid.

#### NEWS ITEMS

HAROLD MILLER, Assistant in Agronomy; W. C. TEMPLETON, Instructor in Farm Crops; and LAWRENCE HENSON, Assistant Agronomist in Forage Crop Investigations, all of the University of Kentucky, are now in the armed forces.

—A—

Dr. M. F. MORGAN, head of the Soils Department of the Connecticut Agricultural Experiment Station at New Haven, was called to active duty as Lieutenant Colonel of Infantry in the United States Army in April. Dr. Herbert A. Lunt will serve as acting head of the Soils Department during Doctor Morgan's absence. Edward Rubins, formerly connected with Rutgers University, has been named Research Assistant in Soils at the Connecticut Experiment Station.

—A—

DR. L. H. ROGERS, Associate Biochemist of the Department of Soils, University of Florida, has been called to active duty in the Chemical Warfare Service of the United States Army.

—A—

DR. JOSEPH CHARLES ARTHUR, formerly head of the Botany Department, Purdue University, died on April 30 at the age of 92. Doctor Arthur was the first botanist of the Indiana Experiment Station, and he and his students made numerous contributions to the knowledge of plant rusts. The Arthur Herbarium contains over 60,000 specimens of plant rusts from all parts of the world.

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### THE AZOTOBACTER PLAQUE TEST AS APPLIED TO THE DETERMINATION OF PHOSPHATE DEFICIENCY IN IDAHO SOILS<sup>1</sup>

W. V. HALVERSEN AND W. G. HOGE<sup>2</sup>

THE problem of rapidly determining fertilizer deficiencies of soils in the laboratory is of great practical importance to agriculture. An expedient method which could be employed to supplement or aid in the interpretation of field tests would be of inestimable value to the farmer and fertilizer industry. Chemical determinations of phosphate in agricultural soils do not reliably differentiate between that which is available to the crop in the field and that fraction which is unavailable. So-called biological methods have been proposed and explored extensively with the hope of overcoming the inherent difficulties of chemical analysis, and varying degrees of reliability have been reported.

Biological methods for determining a fertilizer requirement of a soil depend upon planting samples of the soil with a rapidly growing plant under controlled conditions with and without the particular fertilizer amendment. Comparative yields from the treated and untreated portions of the soil are taken as an index of fertilizer needs. Greenhouse and laboratory tests have employed higher plants, bacteria, such as *Azotobacter*, and certain fungi. Data concerning the amount and type of growth are supplemented where possible by chemical analyses of the plant in order to determine how much of the element in question has been absorbed from the soil and utilized by the plants. The apparent controversial factor in all of these methods is that they are based upon the assumption that the test plant under greenhouse conditions will have the same demand for the nutrients tested as will the crop when growing under natural field conditions.

The *Azotobacter* plaque test for phosphate deficiency had its origin in the experiments of Winogradsky (17, 18, 19, 20),<sup>3</sup> who demonstrated that spontaneous colonies of *Azotobacter* grew luxuriantly

<sup>1</sup>Contribution from the Department of Bacteriology, Idaho Agricultural Experiment Station, Moscow, Idaho. Published with the approval of the Director as Research Paper No. 192. Received for publication August 4, 1941.

<sup>2</sup>Bacteriologist and Assistant Bacteriologist, respectively, Idaho Agricultural Experiment Station.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 511.



upon the surface of soil plaques provided with a suitable source of energy and the essential mineral elements for growth. The methods whereby *Azotobacter* are grown directly on the soil being tested are therefore usually designated as the soil plaque method or the Winoogradsky plaque method. Several European investigators employed various technics of procedure, but Sackett and his coworkers (14, 15) in 1931 and 1932 were the first in the United States to publish comprehensive results from the use of the plaque method for determining phosphate and potash deficiencies in soil. Their report was so favorable for the method that it stimulated many investigators to apply the procedure to routine testing of numerous types of soils throughout the world.

Some investigators have reported the method to be dependable for the determination of phosphate deficiency, while others have considered the test to be unreliable. The most usual criticism of the method has been the conclusion that *Azotobacter* often shows much greater response to phosphate application than do field crops. A discussion of the literature is not presented, but tabulated appraisals by a number of workers who have used the test for determining phosphate deficiencies are summarized in Table 1. The soils employed by these workers are from widely scattered sources and represent a wide range of variation in physical and chemical properties. No attempt is made to classify the soils used because the published reports seldom contained descriptions of the soils. The technics employed, though variable, were fundamentally the same and differed only in minor details. Conflicting opinions regarding the value of the test for determining phosphate deficiency frequently appear to be associated with the chemical and physical properties of the soils tested.

The purpose of this paper is to present data obtained from the application of the *Azotobacter* plaque test to representative Idaho soils.

## METHODS

The specific procedure employed was developed in this laboratory by Halversen.<sup>4</sup> The fundamental technic is similar to that employed by Sackett (14) and the investigators listed in Table 1.

The two important details in which the procedure for the present determinations differed from previous methods are the following: (a) The fertilizer salts used to mix with the soil were diluted in washed 200-mesh silica sand rather than in distilled water. This eliminated moisture adjustments. (b) The treated soil portions ready to be made into plaques were well mixed with mortar and pestle with a small amount of hot, melted agar, and then poured into the testing dishes. When the soil-agar mixture cooled and hardened the "plaque" was artificially "inoculated" with a washed suspension of mixed *Azotobacter* cells. The use of agar greatly diminished the time consumed in preparing the plaques, automatically adjusted the moisture, gave a smooth surface for colony growth, and prevented foaming. The plaques thus prepared held moisture well and were uniform in appearance. The results obtained were noted to be comparable to

<sup>4</sup>Unpublished data.

TABLE I.—*Evaluation of Azotobacter plaque test by various workers.*

Worker	Year	Source of soils	Investigators' appraisal
Winogradsky (18, 19, 20)	1925-27	France	Devised soil plaque technic
Niklas, Scharrer, and Strobel (12)	1926	Germany	Recommended test; application to phosphate testing not emphasized
Ziemiacka (22, 23, 24, 25)	1929-32	Poland and Rothamsted	Reported test successful
Guittonneau (5, 6)	1929	France	Test confirmed by field tests and chemical methods
Walker and Sullivan (16)	1929	Iowa	Test gave encouraging results
Itano and Arakama (7)	1930	Japan	Test not applicable to rice fields
Kryuchkova (11)	1930	Russia	Reported method successful
Sackett and Stewart (14)	1931	Colorado	Recommended test highly
Stewart, Sackett, Robinson, and Kozer (15)	1932	Colorado	Reported Azotobacter test superior to Neubauer and Hoffer cornstalk methods
Dahlberg and Brown (1)	1932	Colorado, Wyoming, Montana, Nebraska	Test gave satisfactory results
Jones (8)	1932	Ontario	Test gave reasonably good correlation with crop returns; minimum phosphate requirements for Azotobacter higher than for growing plants
Pittman and Burnham (13)	1932	Utah	Found test not reliable for soils tested; Azotobacter more responsive to phosphate than most crops
Keller (10)	1932	Germany	Satisfactory results obtained
Greene (4)	1932	Arizona	Correlation with field conditions in only 12% of cases
Young (21)	1933	Utah, Colorado, Iowa	Test of little value for determining phosphate deficiencies in Iowa soils
Joshi and Ayyar (9)	1934	India	Considered minimum phosphate requirements of Azotobacter higher than of crops
Fuller (2, 3)	1934 1935	Massachusetts	Test indicated all soils tested were phosphate-deficient in spite of fact that all produced good crops

those secured when the technic of Sackett (14) was followed exactly.<sup>5</sup> The details of the procedure used are given below.

#### MATERIALS

1. A suspension of mixed cultures of *Azotobacter* cells, washed several times in distilled water by centrifuging.

<sup>5</sup>Unpublished data.

2. Two-hundred-mesh silica sand, washed, and portions prepared as follows: (a) One portion is well mixed with  $K_2SO_4$  so that 100 grams of the mixture contains 15 grams of salt. (b) The second portion is mixed with  $Na_2HPO_4 \cdot 12H_2O$  so that 100 grams of the mixture contains 30 grams of salt. (c) The third portion is mixed with  $K_2HPO_4$  so that 100 grams of the mixture contains 15 grams of the salt.
3. A number of small (about 5 cm $\times$ 2 cm) petri dishes.
4. Hot, melted 2% agar (highest purity) prepared with distilled water.
5. A suitable chamber for keeping the plaques moist while incubating.

#### PROCEDURE

Four 50-gram portions of each soil to be tested are weighed out and to each portion are added 2 grams of Kingsford's cornstarch. To one portion is added 1 gram of the sand containing  $K_2SO_4$  (potassium treatment); to the second is added 1 gram of the sand containing  $Na_2HPO_4$  (phosphorus treatment); to the third is added 1 gram of the sand containing  $K_2HPO_4$  (potassium and phosphorus). The fourth portion depends upon minerals already present in the soil and receives no salt treatment. Each soil portion is then thoroughly mixed with the added salt-sand mixture.

Either the tops or the bottoms of the petri dishes may be used as containers for the plaques. These are labeled on the inside with a wax pencil so that the soil sample and the salt treatment can be later identified. Each of the soil portions is then transferred to a large, warmed mortar and titrated rapidly with 40 cc of the hot, melted agar. This mixture is immediately poured into a labeled half petri dish, which is almost filled. The agar is allowed to solidify and 2 drops of *Azotobacter* suspension are added, spreading this evenly upon the surface. The uncovered plaques are incubated in the moist chamber for 72 hours at about 28° C.

#### INTERPRETATION OF RESULTS

After the incubation period each sample that contains suitable nutrients will have developed *Azotobacter* growth on the agar surface. The growth varies from waxy white to dark brown, is moist and glistening, and is easily discernible with the unaided eye. Due to the method of artificial "inoculation" discreet colonies rarely appear.

The comparative amount of growth occurring on the phosphate-treated and check plaques serves as an indication of the degree of the deficiency in the soil. It is convenient to record data in tabular form, using the following symbols: 0=no growth; + =slight growth; ++ =moderate growth; +++ =good growth; ++++ =excellent growth.

Data obtained in this manner can be used to indicate potassium deficiency as well as phosphorus deficiency, but no attempt is made in this report to consider potassium deficiency. The use of potassium salts does serve to rule out instances where a potassium deficiency could obscure a lack of growth due to the absence of phosphorus. Table 3 interprets the degree of phosphate deficiency according to the data from the plaques.

Soils more acid than pH 5.8 do not support the growth of *Azotobacter* and therefore require the addition of 10% of  $CaCO_3$  if they are to be tested by this method. However, results obtained with such soils have not been encouraging.

## DISCUSSION OF RESULTS

The data in Table 2 prove the necessity for artificially "inoculating" the plaques with a washed *Azotobacter* cell suspension. Many of the plaques prepared without inoculation developed few or no spontaneous *Azotobacter* colonies, whereas excellent growth occurred where artificial "inoculation" was employed. One hundred and nineteen samples of soil were tested both with and without artificial inoculation. Where no "inoculation" was employed, 34% of the samples were interpreted as not deficient in phosphorus and 66% were classified as slightly deficient to very deficient. In contrast, artificially "inoculated" plaques showed 14% not deficient while 86% were classified as slightly deficient to very deficient. These results confirm data of several previous investigators who have recommended artificial inoculation of the plaques.

TABLE 2.—*Phosphate deficiency of representative Idaho soils according to the Azotobacter plaque test.*

Degree of phosphate deficiency	A, artificially "inoculated" plaques vs. spontaneous <i>Azotobacter</i> plaques, 119 samples				B, results for 425 samples	
	Artificially inoculated		Spontaneous growth		Artificially inoculated	
	Number	%	Number	%	Number	%
Not deficient.....	17	14	40	34	71	17
Slightly deficient.....	6	5	37	31	73	17
Moderately deficient...	8	7	17	14	56	13
Substantially deficient	36	30	17	14	119	28
Very deficient.....	52	44	8	7	106	25

Table 2, B summarizes data on phosphate deficiency for artificially "inoculated" plaques made from 425 representative Idaho soils. These data show that 83% of the soils tested ranked slightly deficient to very deficient in phosphorus. Crop yield data are not available for these particular soils to serve for interpretation. However, since most of these soils are productive, it is believed that the test indicated deficiency in many cases where the supply was adequate for field crops. Certainly, 66% of these soils should not be classified as moderately deficient to very deficient in phosphorus.

Thirty-eight samples of soil for which yield data were available<sup>6</sup> show the responses of alfalfa, potatoes, and beets to superphosphate fertilization were tested by the procedure described. Table 3 contains the results and interpretation given to the tests, while Table 4 gives the results of field tests where phosphate was added and compares data obtained by field testing with data obtained with the Azoto-

<sup>6</sup>The yield data and method of evaluation of crop response due to fertilization for these soils and the actual samples of the soils tested were kindly supplied by the Agronomy Department of the University of Idaho through the courtesy of Prof. G. O. Baker.

TABLE 3.—Results and interpretation of *Azotobacter* plaque tests.

Soil No.	Check plaque	Salts added*			Indicated phosphorus deficiency
		Na <sub>2</sub> HPO <sub>4</sub>	K <sub>2</sub> SO <sub>4</sub>	K <sub>2</sub> HPO <sub>4</sub>	
3186	o	++++	o	++++	Very deficient
3589	o	+++	o	+++	Substantially deficient
3689	o	+++	o	+++	Substantially deficient
3382	o	+++	o	+++	Substantially deficient
3497	+	o	o	++	No interpretation possible
3146	o	+++	++++	+++	Substantially deficient
3962	o	+++++	o	+++++	Very deficient
3532	o	+++	o	+++	Substantially deficient
3551	+	++++	+	++++	Substantially deficient
3177	o	+++	o	++++	Substantially deficient
3231	o	o	o	+++	Moderately deficient
3148	o	+	o	+++	Moderately deficient
3544	o	++++	o	++++	Very deficient
3152	o	++	o	+++	Moderately deficient
3553	o	++++	o	++++	Very deficient
3238	o	++	o	+++	Moderately deficient
3208	o	++++	o	++++	Very deficient
3247	o	++	o	++	Moderately deficient
3500	o	++	o	++++	Substantially deficient
3795	o	++++	o	++++	Very deficient
3356	o	+++	+	++++	Substantially deficient
3640	o	++++	o	++++	Very deficient
3825	o	++	o	++++	Substantially deficient
3637	o	+++	o	+++	Substantially deficient
3636	o	++++	o	++++	Very deficient
3151	+	+++	+	+++	Moderately deficient
3144	o	++++	o	++++	Very deficient
3646	o	+++	o	+++	Substantially deficient
3545	+	++++	+	++++	Substantially deficient
3456	o	+	o	++	Slightly deficient
3641	o	++	o	++	Moderately deficient
3606	o	++	o	++	Moderately deficient
3633	o	++	o	+++	Moderately deficient
3427	o	++	o	++	Moderately deficient
3628	o	++++	o	++++	Very deficient
3502	o	++	o	++++	Substantially deficient
3176	o	++++	o	++++	Very deficient
3598	o	++++	o	++++	Very deficient

\*o = no growth; + = slight growth; ++ = moderate growth; +++ = good growth; ++++ = excellent growth.

bacter plaque test. The cash value of any increased yield resulting from phosphate fertilization was divided by the cost of the phosphate application as a means of interpreting the field tests. The factors thus obtained served as an empirical method of stating phosphate deficiency from the practical standpoint. Where the factor was greater than 1, the soil was considered deficient in phosphorus; the greater the factor, the greater the deficiency. Where the factor was greater than 3, the deficiency was considered economically significant. It is acknowledged that these factors have only an empirical interpretation due to constant fluctuation in market value of the crops. Potatoes were evaluated at \$0.50 per 100 pounds, wheat at \$0.60 per bushel,

TABLE 4.—Comparison of crop response in field tests with *Azotobacter* plaque tests.

Soil No.	Phosphate rate per acre	Increase in yield per acre	Gain/cost factor*	Phosphate deficiency according to plaque test
Wheat				
3186	50	-1.6	-0.8	Very deficient
Alfalfa				
3589	200	1.3	1.6	Substantially deficient
3689	200	-0.3	-0.4	Substantially deficient
3382	200	-0.2	-0.2	Substantially deficient
3497	200	0.7	0.8	?
3146	200	0.8	1.0	Substantially deficient
3962	200	0.4	0.5	Very deficient
3532	200	0.6	0.7	Substantially deficient
3551	150	1.6	3.0	Substantially deficient
3177	125	-0.4	-0.6	Substantially deficient
3231	125	0.6	1.2	Moderately deficient
3148	125	1.2	2.2	Moderately deficient
3544	100	0.7	1.7	Very deficient
3152	100	0.0	Not deficient	Moderately deficient
3553	100	0.3	0.7	Very deficient
Potatoes				
3238	400	23.1	1.6	Moderately deficient
3208	125	17.6	2.8	Very deficient
3247	125	6.8	1.0	Moderately deficient
3500	125	35.0	5.6†	Substantially deficient
3795	125	35.8	5.7†	Very deficient
3356	100	193.0	38.6†	Substantially deficient
3640	100	18.5	3.7†	Very deficient
3825	100	45.5	9.1†	Substantially deficient
3637	100	15.5	3.1†	Substantially deficient
3636	100	0.0	Not deficient	Very deficient
Beets				
3151	200	2.3	2.8	Moderately deficient
3144	200	1.7	2.0	Very deficient
3646	150	2.6	4.2†	Substantially deficient
3545	125	5.2	10.0†	Substantially deficient
3456	125	1.5	2.9	Slightly deficient
3641	125	3.3	6.3†	Moderately deficient
3633	125	-0.3	-0.6	Moderately deficient
3606	100	0.0	Not deficient	Moderately deficient
3427	100	-0.8	-1.9	Moderately deficient
3528	100	3.6	8.6†	Very deficient
3502	100	0.2	0.5	Substantially deficient
3176	100	1.0	2.4	Very deficient
3598	100	3.4	8.2†	Substantially deficient

\*Value of crop increase/cost of phosphate application.

†Considered phosphate-deficient from economic standpoint.

beets at \$6.00 per ton, and alfalfa hay at \$6.00 per ton for the purpose of ascertaining the value of the crop increments. The average cost of the phosphate employed was \$2.50 per 100 pounds.

An analysis of the results secured by the plaque method of testing would indicate that 36 of the 38 soils would be considered moderately

deficient to very deficient in phosphorus, while the analysis of field data reveals that only 11 of the 38 soils were deficient to the extent that phosphate applications were justified from the economic standpoint. If a more liberal interpretation of phosphorus deficiency according to field tests were employed and all soils considered deficient where the factor *value of yield increment/cost of phosphate application* was 1 or greater, 24 of the 38 soils would be considered deficient. The latter interpretation is probably not justified from an economic point of view, though the value of the increase in yield would be equal to or greater than the cost of phosphate fertilization. There is no correlation with the *degree of deficiency* as shown by crop yields with the *degree of deficiency* as shown by plaque tests.

In view of the data presented it would appear that the plaque test, when applied to representative Idaho soils, is of doubtful value in determining whether or not phosphate should be applied. Azotobacter respond to the addition of phosphate salts in the plaque test where field crops fail to respond significantly to applications of superphosphate. Therefore, the plaque test would indicate the need for phosphate fertilization in a great many soils where phosphate application would not be economically justified.

#### SUMMARY

The modified Azotobacter plaque test used in this work offers several advantages over procedures commonly followed by previous investigators as follows: (a) The substitution of agar solution in place of water supplies a smooth, moist surface for the growth of the organisms; (b) foaming and difficulties due to moisture and physical differences of soils are overcome; (c) the dilution of fertilizer salts with 200-mesh silica sand facilitates the measuring out of small quantities with a high degree of accuracy and eliminates the necessity of making moisture adjustments; (d) the authors confirm the results of previous investigators who have recognized the necessity of artificial "inoculation" of soil plaques with washed Azotobacter cells.

The modified plaque test was applied to 425 representative Idaho soils of which 17% were classified as not deficient, 83% ranged from slightly deficient to very deficient, and 66% ranked from moderately deficient to very deficient. Since most of these soils are representative productive soils of Idaho, it seems unlikely that 66% should logically be classified as moderately to very deficient in phosphorus.

Data on 38 soil samples on which crop yield data were available to establish the value of phosphate fertilization are presented. According to the yield data, phosphate application was considered as justified from an economic standpoint in 11 of the 38 soils, whereas the modified Azotobacter plaque test indicated that 36 of the 38 soils were definitely phosphate deficient. Therefore, it is evident that, although the modified Azotobacter plaque procedure overcomes certain difficulties encountered in the previously reported studies, it is not a reliable indicator of phosphate deficiency in the Idaho soils tested, because Azotobacter growth indicated much greater response

to phosphate when added in the plaque test than did field crops to normal fertilization with phosphate under field conditions.

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## THE DISSEMINATION OF PRICKLY PEAR SEED BY JACK RABBITS<sup>1</sup>

F. L. TIMMONS<sup>2</sup>

RECENT experiments at the Fort Hays Branch of the Kansas Agricultural Experiment Station indicate that jack rabbits are important agents in disseminating the seed of prickly pear cactus. It may be responsible for much of the rapid spread of this weedy plant over the range lands in western Kansas and possibly in other sections of the Great Plains and Southwest in recent years. The increased attention now being given to the control of prickly pear encourages consideration of this method of spreading the seed.

Dameron and Smith (1)<sup>3</sup> estimated that the area in Texas infested with prickly pear (*Opuntia* spp.) comprises at least 60 million acres but stated that it is only during recent years that the plant has become sufficiently abundant to give concern to livestock men. Nearly 4 million acres of pasture land in Kansas are infested with prickly pear according to a survey made by Yost (8) in 1939.

The importance of rabbits and other animals as agencies in the spread of prickly pear (*Opuntia* spp.) was recognized in Australia (2) in 1919. Most of the seeds found in rabbit droppings were chewed into small bits but occasionally whole seeds were observed. The emu and the black magpie were considered of much more importance in the spread of prickly pear since they ate large quantities of the ripened fruit and the seed passed uninjured through their digestive tracts and was scattered by them over wide areas. Large quantities of seed were also found in the droppings of cattle.

Toit (3) and Phillips (4) reported that in South Africa the fruit of prickly pear (*Opuntia* spp.) furnishes an important item in the diet of native tribesmen and that seed of increased viability is thus disseminated over wide areas. Monkeys, baboons, goats, and various kinds of birds were also reported to play important roles in disseminating the seed. Cattle were observed to eat ripened prickly pear fruits avidly, often acquiring such a craving for them that they would eat practically nothing else during the fruiting season.

At Hays, Kans., the activities of jack rabbits in feeding upon ripened fruit and in disseminating seed of prickly pear were observed (7) by chance in August 1939 during the course of some cactus eradication experiments. Following this observation, rather careful studies were made to determine the nature and extent of the seed dissemination. Germination tests of seed recovered from rabbit droppings and from the dried fruits were made to determine the effect upon viability of passage through the digestive tract of the jack rabbit.

<sup>1</sup>Contribution of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, in cooperation with the Department of Agronomy, Kansas Agricultural Experiment Station, Hays, Kans. Contribution No. 33 Fort Hays Branch Station. Received for publication August 16, 1941.

<sup>2</sup>Associate Agronomist. The writer is particularly indebted to L. E. Wenger, Agent, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, and Forage Crops Specialist, Fort Hays Branch Agricultural Experiment Station for many helpful suggestions and supplementary observations in connection with the study reported here.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 520.

## OBSERVATIONS ON RANGE LAND AT HAYS, KANS.

The observations at Hays were made in pasture areas infested with 150 to 200 prickly pear plants to the acre. *Opuntia macrorrhiza* Engelm. was the prevalent species, although plants of *O. tortispina* were also present. Jack rabbits (*Lepus californicus melanotis*) were present in the vicinity in about normal numbers during the study but were much less numerous than in some recent years, notably in 1935.

*Opuntia macrorrhiza* has a somewhat branched prostrate stem and the terminal internodes and fruits rarely attain a height of more than 8 or 10 inches. The ripened fruit is fleshy, juicy, has an attractive reddish purple color and a pleasant fruity taste, and is free from spines. The flattened disc-like seeds are contained in the pulpy mass of the fruit and apparently are swallowed by rabbits when eating the fruit. *O. tortispina* has similar characteristics.

Jack rabbits were observed to begin feeding upon the fruit of prickly pear early in August, both in 1939 and 1940, and to continue until after the fruit had dropped off following the first severe freeze in November (Fig. 1). Some feeding apparently took place even later in the season after the fruit had dropped. During the fall and winter months most of the recently deposited rabbit droppings found in pastures infested with prickly pear contained 1 to 15 undamaged seeds in each pellet (Fig. 2).

Two collections of jack rabbit pellets were made at random in a prickly pear infested pasture, the first on February 13, 1940, and the second January 7, 1941. The method of collecting was to walk across the infested pasture in a more or less straight course, stopping every

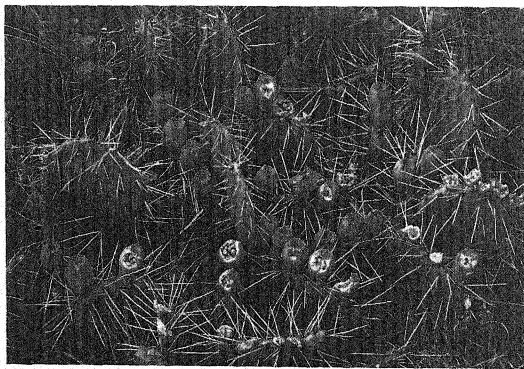


FIG. 1.—A close-up view of a portion of a plant of prickly pear (*Opuntia macrorrhiza*) showing evidence of feeding upon the ripened fruits by jack rabbits. Note the pulpy mass containing seed exposed in the partly eaten fruits.

few rods and collecting all of the pellets within reach. Several trips were made across the pasture in order to provide an adequate sampling of the entire area. The pellets were examined individually and counts made of the number of undamaged prickly pear seeds found in each. The results from the two studies are presented in Table 1.

TABLE 1.—*The frequency of occurrence of different numbers of prickly pear seeds in jack rabbit droppings collected at random on range land at Hays, Kans.*

Number of seeds per pellet	Pellets collected Feb. 13, 1940		Pellets collected Jan. 7, 1941	
	Number of pellets	Total number seeds	Number of pellets	Total number seeds
0	242	0	86	0
1	65	65	36	36
2	70	140	28	56
3	56	168	25	75
4	41	164	25	100
5	30	150	12	60
6	25	150	12	72
7	18	126	8	56
8	15	120	4	32
9	6	54	2	18
10	2	20	2	20
11	4	44	8	88
12	3	36	3	36
13	0	0	0	0
14	3	42	0	0
15	2	30	2	30
Totals	582	1,309	253	679

Approximately 59% of the pellets collected February 13, 1940, and 66% of those collected January 7, 1941, contained one or more undamaged prickly pear seeds, the number ranging from 1 to 15 in the individual pellets. The average number of seeds in each pellet was 2.2 in the 1940 collection and 2.7 in those collected in 1941.

It should be pointed out that all of the pellets examined in this study were collected not more than 2 rods from prickly pear plants. Taylor, Vorhies, and Lister (6) state that jack rabbits regularly defecate as they feed, or very soon afterwards. Therefore, it is probable that a large proportion of the seeds consumed by jack rabbits which fed regularly on prickly pear fruits in this pasture also were dropped in the pasture. However, jack rabbit pellets containing prickly pear seed were found rather frequently on experimental weed control plots and on native grass revegetation areas located as far as  $\frac{1}{4}$  mile from the infested pasture and any seed-bearing plants. The percentage of pellets containing seed found in these areas was much lower than that of pellets collected in the infested pasture land, but the number of seeds was sufficient to indicate that jack rabbits disseminate the seed of prickly pear in quantities over a considerably wider area than the immediate vicinity in which they feed upon the fruit. Prickly pear seedlings have been observed emerging singly and

in clusters of several seedlings every year since 1935 on chemically treated bindweed control plots at distances of  $\frac{1}{8}$  to  $\frac{1}{4}$  mile from a cactus-infested native grass pasture.

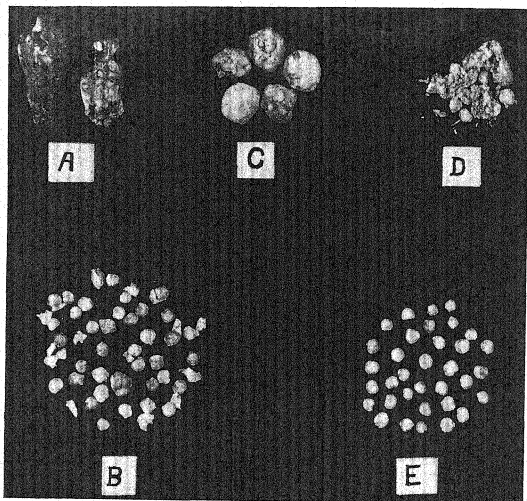


FIG. 2.—A, dried prickly pear fruits containing mature seed; B, seeds threshed from dried fruit with adhering dried pulp; C, jack rabbit droppings containing prickly pear seeds; D, a crushed pellet showing several prickly pear seeds; E, prickly pear seeds recovered from jack rabbit droppings. Note the freedom from adhering pulp.

Very few damaged prickly pear seeds were found in the rabbit pellets as contrasted with the observation of Darnell-Smith (2) who reported that in Australia most of the seeds found in rabbit droppings were chewed into small pieces. Apparently the seeds of *Opuntia macrorrhiza* and the other species involved in this study are so hard when mature that they are generally uninjured by the mastication of the ripened fruit by jack rabbits.

#### SEED GERMINATION TESTS

Three collections of prickly pear seed were made from rabbit droppings and also from mature pears in the pasture under observation. Seeds produced in 1939 were collected on October 25, 1939, and

February 13, 1940, and those produced in 1940 were collected on January 7, 1941. A total of six germination tests were made during 1940 and 1941 to compare the viability of seed recovered from jack rabbit droppings with that of normal seeds taken from dried mature fruit.

The seed was planted in soil in a greenhouse flat in each test. The seed from jack rabbit droppings and from dried mature fruit were placed in alternate rows in the flats. The flats in lots II, III, and V were kept in a warm greenhouse continuously, while those in lots I, IV, and VI were exposed to winter temperatures. During the periods of exposure the minimum daily temperatures ranged mostly below freezing to as low as 11°F, while the maximum daily temperatures ranged mostly above freezing to as high as 79°F. After the periods of outdoor exposure the flats were returned to the greenhouse where germination occurred. The soil was kept moist in all flats during the entire experiment.

The results of the germination tests are given in Table 2. Germination did not begin until 5 to 6 weeks after the flats were placed in the greenhouse and continued until 7 to 10 weeks in all except lot VI in which germination started in 2 weeks and was complete after 4 weeks.

The seeds recovered from rabbit droppings germinated 7%, while those taken from ripened fruit showed only a trace of germination when tested prior to winter exposure. Exposure to winter weather in moist soil for periods of 3 to 6 weeks increased the average germination of seeds taken from rabbit droppings to 62% and of those taken from dried fruit to 44%. Thus, the digestive action of the jack rabbit not only failed to injure the viability of prickly pear seed but improved the immediate germination approximately 50% on the average. This is in agreement with the observations of Toit (3) and Phillips (4) in South Africa where the viability of prickly pear seed was extremely low when taken directly from the fruit but was greatly increased after passing through the digestive tract of an animal.

The seed collected from the pasture February 13, 1940, and January 7, 1941, had been exposed to severe winter weather, but the immediate germination was very low, probably due to the fact that the seeds had been lying on the surface in the relatively dry rabbit droppings or dried fruit. When such seeds are covered with soil by natural means and then subjected to the action of winter weather with ample moisture present, the improvement in germination probably is comparable to that obtained in the greenhouse flats when they were exposed in moist soil to outside winter temperatures. Riegel (5) has observed that ground squirrels (*Citellus tridecemlineatus*) gather the prickly pear seed from jack rabbit droppings and store them for future use as food in caches just below the surface of the ground. He found clusters of prickly pear seedlings arising from 13 such caches during one season in an area of approximately 2 acres. Thus, the ground squirrel apparently serves as an important agent for burying the seed in soil where the presence of moisture and the action of low temperatures during the winter results in a considerable percentage of germination.

TABLE 2.—*Effect on germination of passage of prickly pear seed through the digestive tracts of jack rabbits.*

Lot No. and date seeds were collected	Treatment of seed previous to and during germination test	Percentage germination		
		Days after flat placed in greenhouse	Seeds from jack rabbit droppings	Seeds from dried mature fruit
1939 Seed				
Lot I Oct. 25, 1939	Exposed to outdoor winter temperatures Nov. 10 to Dec. 23, 1939; placed in greenhouse Dec. 23	44 58 70*	40 75 81	11 42 57
Lot II Feb. 13, 1940	Planted in soil Feb. 27, 1940, and kept in warm greenhouse	34 70*	2 9	0 0
Lot III Feb. 13, 1940	Planted in soil Jan. 3, 1941, and left in warm greenhouse	25 48*	4 12	0 0
Lot IV Feb. 13, 1940	Planted in soil Feb. 20, 1941, and exposed out-of-doors until March 15, then placed in greenhouse	39 55 68*	4 57 74	1 58 65
1940 Seed				
Lot V Jan. 7, 1941	Planted in soil Jan. 17, 1941, and left in warm greenhouse	41 50*	0 0	1 1
Lot VI Jan. 7, 1941	Placed out-of-doors March 8, 1941, and returned to warm greenhouse March 31	14 21 29*	7 24 31	0 5 10
Average of final readings in all lots.....		—	34.5	22.2
Average of final readings in lots II, III, and V (tested prior to winter exposure).....		—	7.0	0.3
Average of final readings in lots I, IV, and VI (tested after winter exposure).....		—	62.0	44.0

\*Time at which final count was made.

## DISCUSSION

The conditions in the pastures in which the observations reported here were made are typical of those on range land throughout western Kansas with respect to the species of cactus; the type, stand, and vigor of native grass cover; and the number of jack rabbits present. It appears logical to conclude that jack rabbits have been an important means of disseminating the seed of prickly pear throughout this region where low-growing species with fleshy unarmed fruits are predominant. Probably jack rabbits are not important agents in spreading the seed of tall-growing species of prickly pear or of low-growing species that have dry or spiny fruit.

Cattle, sheep, and goats are undoubtedly responsible in many cases for the spread of prickly pear in the Great Plains and Southwest regions of this country just as they have been reported to be in Australia and South Africa. Rodents and possibly certain kinds of birds may also be involved in the spread of the seed.

Jack rabbits were evidently the most important agents in the dissemination of the seed during the alarming spread of prickly pear to previously noninfested pastures and vicinities and intensification of the infestation in previously infested areas that occurred in western Kansas during the years immediately following 1935 when jack rabbits were very numerous and numbers of livestock on ranges were greatly reduced. In that year "rabbit drives" were organized and many thousands of jack rabbits were killed in nearly every county in western Kansas in order to save growing crops and feed supplies. Under conditions of extreme food shortage, it is probable that jack rabbits consume nearly all of the ripened prickly pear fruits and disseminate the seeds generally over a region in their migrations from one area to another.

No study was made of the extent to which the seed disseminated by jack rabbits actually develop plants under range conditions, but it is known that the species of *Opuntia* which occur in Kansas are spread chiefly by means of seeds. Undoubtedly the extreme drought conditions and the consequent reduction in vigor and stand of grass during the period from 1934 to 1939 were important factors contributing to the ability of the slow-growing prickly pear seedlings to establish themselves. Under more favorable range conditions it is doubtful if many prickly pear seedlings are able to survive the competition of a vigorous growth of grass and the trampling of grazing livestock. Probably the most practical means of preventing the spread of prickly pear from seed is the proper management of range land to promote the maximum stand and vigor of grass. Nevertheless, because of the likelihood of the recurrence of dry years, eradication of established cactus as soon as possible would seem to be advisable in order to eliminate the source of seed and thus prevent its spread by jack rabbits or other means.

#### SUMMARY

The occurrence of prickly pear seeds in the droppings of jack rabbits that had fed upon the ripened fruits was determined from collections made in and near a cactus infested pasture at Hays, Kans.

The droppings collected in the pasture contained an average of about 2.5 seeds per pellet, while those collected in nearby uninfested fields only occasionally contained seeds.

The germination of the seeds found in the jack rabbit droppings was about 50% greater than that of seeds taken from dried fruits at the same time.

Jack rabbits evidently are important agents in the dissemination of viable prickly pear seed and presumably in the spread of prickly pear plants through western Kansas pastures.



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# A METHOD OF STATISTICAL ANALYSIS OF A FACTORIAL EXPERIMENT INVOLVING INFLUENCE OF FERTILIZER ANALYSES AND PLACEMENT OF FERTILIZER ON STAND AND YIELD OF CANNERY PEAS<sup>1</sup>

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IN the attempt by investigators to increase the efficiency of field experiments, a number of complicated experimental designs have been suggested by which the amount of information that may be secured from an experiment is measurably increased. However, the statistical analysis of the data resulting from such an experiment likewise becomes more involved and misuse of statistical methods is more likely to occur. This paper is presented with the idea that agronomists would be interested in a detailed statistical analysis and interpretation of results of a "factorial" design experiment concerning fertilizers on cannery peas.

The purpose of this experiment was to study the effect of different fertilizer mixtures, placed in various locations with respect to the seed, on the yield and stand of cannery peas. Of special interest was the method for obtaining the interaction between fertilizers and placements. In an experiment of this kind it is necessary for reasons associated with the practicability of mechanical operations in conducting field experiments to limit the number of treatments to a minimum.

The number of plots in an experiment with cannery peas is especially important because the quality of the peas changes very rapidly at harvest time and prices are dependent on quality. It is important, then, to have an experimental design which will furnish the desired information and still include no more plots than can be harvested within a 36-hour period. Because of this limit on the number of plots which could be cared for, the fertilizers were limited to three, 0-20-0, 0-16-8, and 4-16-8. The placements were also limited to three in number: Placement 1, fertilizers applied in contact with the seed; placement 2, fertilizers applied in bands  $\frac{1}{2}$  inch out and  $1\frac{1}{2}$  inches below the seed; placement 3, fertilizers applied in bands 2 inches out and  $1\frac{1}{2}$  inches below the seed.

The rate of application was 300 pounds per acre for the 4-16-8 and the 0-16-8 analyses and 240 pounds per acre for the 0-20-0. This arrangement provided for equivalent quantities of phosphate on all plots receiving fertilizer. By the use of three fertilizers and an unfertilized control and three methods of placement there are 12 treat-

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ments possible in this design. This means, however, that in three different treatments, one for each method of placement, no fertilizer is applied. The plots included in these three treatments where, theoretically, "no fertilizer" was applied in three different locations are, in reality, dummy plots. This situation arises because the placements were so similar that differences in mechanical disturbance of the soil were not great enough to affect the yield significantly. Any differences in yield, then, among the treatments which did not include fertilizer were due to experimental error.

This experiment was located on a Brookston loam soil in Huron County. The planting was made with a grain drill equipped by the Bureau of Agricultural Chemistry and Engineering of the U. S. Dept. of Agriculture to place the fertilizer in various locations with respect to the seed. The plots were arranged in five randomized blocks and were 100 feet long and two drill widths wide. The field arrangement is outlined in Table 1, together with stands for each plot.

The statistical analysis of the data begins with the usual calculation of the total sum of squares shown in Table 1. Since the procedure for stand and yield data is exactly the same, the presentation of a detailed statistical analysis is limited to the stand data.

The next step in the analysis is the preparation of Table 2. All data from unfertilized plots are grouped together in accordance with the technic involved in handling dummy plots. In this table the stand figure used for the unfertilized plots consists of the total for the three plots in each block. The total sum of squares for Table 2 is the sum of the squares of each individual plot number for the nine treatments plus the sum of the squares of the totals for the unfertilized plots divided by three, the number of items in each no treatment plot total, minus the correction term  $C_1$ . The technic for obtaining the total sum of squares for data in Table 2, together with sums of squares due to blocks, treatments, and error, are given below the table.

It is necessary to separate the sum of squares due to variations between dummy plots from the other sums of squares. If one breaks up the total sum of squares into sums of squares due to blocks and treatments and then subtracts the sum of the last two sums of squares from the total sum of squares to obtain an error sum of squares, the variations due to dummy plots will be included in the error sum of squares. The sum of squares due to variations between dummy plots is not part of the error sum of squares and should be singled out. This sum of squares is called the sum of squares "within blocks." The error is found from the sum of squares due to the interaction of blocks and treatments. (For a discussion of the use of dummy plots see "Designs of Experiments" by F. A. Fisher.) The procedure used in calculating the "within blocks" sum of squares due to dummy plots is presented in Table 4.

The treatment totals for the nine treatments include 5 items, whereas the total for the unfertilized plots is obtained from 15 items, thus necessitating the procedure used for obtaining the sum of squares for treatment. The analysis indicating the degrees of freedom and sum of squares is included in Table 2, with degrees of freedom as follows: Total 49; between blocks 4; between treatments 9; treatments  $\times$

TABLE 1.—Field arrangement of plots and stands.

Block 1	$\frac{1}{2}$ in. out 0-20-0 215	2 in. out none 260	$\frac{1}{2}$ in. out none 225	2 in. out 0-20-0 261	Contact 4-16-8 169	Contact 0-16-8 232	2 in. out 0-16-8 248	$\frac{1}{2}$ in. out 4-16-8 237	$\frac{1}{2}$ in. out 0-16-8 255	Contact 0-20-0 239	Contact none 253	2 in. out 4-16-8 247
Block 2	Contact none 165	$\frac{1}{2}$ in. out 4-16-8 195	$\frac{1}{2}$ in. out none 227	$\frac{1}{2}$ in. out 0-16-8 224	Contact 0-16-8 178	2 in. out 4-16-8 242	2 in. out 0-16-8 243	$\frac{1}{2}$ in. out 0-20-0 228	2 in. out 0-20-0 284	2 in. out none 268	Contact 4-16-8 163	Contact 0-20-0 240
Block 3	$\frac{1}{2}$ in. out 0-16-8 155	$\frac{1}{2}$ in. out 4-16-8 204	$\frac{1}{2}$ in. out none 248	2 in. out 0-16-8 264	2 in. out 0-20-0 243	2 in. out 4-16-8 237	2 in. out none 283	Contact 0-16-8 266	$\frac{1}{2}$ in. out 0-20-0 269	Contact none 266	Contact 0-20-0 253	Contact 4-16-8 231
Block 4	$\frac{1}{2}$ in. out none 195	Contact 0-20-0 211	$\frac{1}{2}$ in. out 0-16-8 253	Contact 0-16-8 239	$\frac{1}{2}$ in. out 0-20-0 262	$\frac{1}{2}$ in. out 4-16-8 270	2 in. out 0-20-0 250	2 in. out none 261	2 in. out 0-16-8 283	Contact none 304	Contact 4-16-8 197	2 in. out 4-16-8 228
Block 5	$\frac{1}{2}$ in. out none 197	$\frac{1}{2}$ in. out 0-16-8 268	2 in. out 0-16-8 261	Contact 0-16-8 247	$\frac{1}{2}$ in. out 0-20-0 252	Contact 4-16-8 159	Contact 0-20-0 221	2 in. out 4-16-8 283	2 in. out none 265	$\frac{1}{2}$ in. out 4-16-8 270	Contact none 302	2 in. out 0-20-0 233
Total												14,338

Rate per acre, 300 lbs. for 4-16-8 and 0-16-8 and 240 lbs. for 0-20-0.  
 Placements,  $\frac{1}{2}$  in. to the side and  $1\frac{1}{2}$  in. below the seed; 2 in. to the side and  $1\frac{1}{2}$  in. below seed; and, contact with the seed.

Correcting term,  $C = \frac{60}{(14,338)^2} = 3,426,304$ .

Grand total sum of squares =  $(215)^2 + \dots + (302)^2 - C = 3,492,464 - 3,426,304 = 72,160$ .

TABLE 2.—*Procedure used in determining sums of squares due to blocks, treatments, and error (plants per 40 feet of row).*

Treatments	Blocks					Total	Mean
	1	2	3	4	5		
1/2 in. out 0-20-0...	215	228	269	262	252	1,226	245
1/2 in. out 0-16-8...	255	224	155	253	268	1,155	231
1/2 in. out 4-16-8...	237	195	204	270	270	1,176	235
2 in. out 0-20-0...	261	284	243	250	233	1,271	254
2 in. out 0-16-8...	248	243	264	283	261	1,299	261
2 in. out 4-16-8...	247	242	237	228	283	1,237	247
Contact 0-20-0...	239	240	253	211	221	1,164	233
Contact 0-16-8...	232	178	266	239	247	1,162	232
Contact 4-16-8...	169	163	231	197	159	919	184
None.....	748	660	797	760	764	3,729	248
Total.....	2,851	2,657	2,919	2,953	2,958	14,338	

Total sum of squares =

$$(215)^2 + \dots + (159)^2 + \frac{(748)^2 + \dots + (764)^2}{3} - C_1 = 3,479,879 - 3,426,304 = 53,575.$$

Sum of squares between blocks =

$$(2,850)^2 + \dots + (2,958)^2 - \frac{41,178,384}{12} - C_1 = 5,228$$

Sum of squares between treatments =

$$(1,226)^2 + \dots + (919)^2 + \frac{(3,729)^2}{15} - C_1 = 3,447,587 - 3,426,304 = 21,283$$

Source	D. F.	Sums of squares
Total	49	53,575
Between blocks	4	5,228
Between treatments	9	21,283
Treatments $\times$ blocks	36	27,064

Difference for significance between stand means = 35.0

Difference for significance between any treatment mean and mean of plots receiving no fertilizer = 28.7

blocks 36. The sum of squares of treatments  $\times$  blocks is the error term to be used in the final analysis.

The next step in the procedure is to break down the sum of squares for treatments into its components since this sum of squares includes all of the variation due to treatments. The value of a factorial experiment lies chiefly in the efficiency of the design to enable one to allocate correctly differences due to the various factors entering into treatment variation. In this experiment the variation due to treatment is ascribed to three sources, namely, the variation resulting from the effect of fertilizers, the effect due to fertilizer placement, and the effect due to the interaction between fertilizers and placement. The sums of squares for these are given in Table 3. For example, the value for the 0-20-0 treatment in any block is made up of the stand count for the "0-20-0, contact" + "0-20-0, 2 inches out" + "0-20-0, 1/2 in out". In this experiment it is not possible to segregate the variance due entirely to fertilizer since there are no plots in which fertilizer is separated from placement. To clarify this point, this example can be cited. The data in Table 3 indicate that a 4-16-8 fertilizer

adversely affects the stand. However, this effect on stand is not due entirely to the fertilizer analysis but partly to the placement of the fertilizer, since if the 4-16-8 fertilizer is not applied in contact with the seed, no injury to stand will result (Fig. 1).

The next step in the analysis is to determine what part of the sum of squares due to treatment can be ascribed to the placement of the fertilizer. This part of the procedure is presented in part of Table 3. In addition to the effect of placement it is also desirable to know whether or not one fertilizer will cause more or less injury to the germination than one of a different analysis applied in the same manner. This information is also obtained from Table 3 in the interaction between fertilizers and placements. In this table, it was

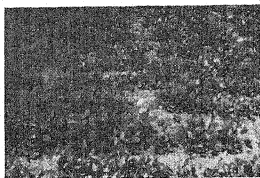


FIG. 1.—Stand of cannery peas on a plot receiving 300 pounds of a 4-16-8 fertilizer applied  $\frac{1}{2}$  inch to the side and  $1\frac{1}{2}$  inches below the seed.

TABLE 3.—*Procedure for determining the sums of squares due to placement, fertilizers, and the interaction between fertilizers and placements.*

Fertilizers	Placements			Total
	$\frac{1}{2}$ in. out	2 in. out	Contact	
0-20-0	1,226	1,271	1,164	3,661
0-16-8	1,155	1,299	1,162	3,616
4-16-8	1,176	1,237	919	3,332
Total . . . . .	3,557	3,807	3,245	10,609
None . . . . .				3,729

$$C_2 = \frac{(10,609)^2}{45} = 2,501,131$$

$$\text{Total sum of squares} = \frac{(1,226)^2 + \dots + (919)^2}{5} - C_2 = 2,520,557 - 2,501,131 = 19,426$$

$$\text{Sum of squares between placements} = \frac{(3,557)^2 + (3,807)^2 + (3,245)^2}{15} - C_2 = 2,511,702 - 2,501,131 = 10,571$$

$$\text{Sum of squares between fertilizers} = \frac{(3,661)^2 + (3,616)^2 + (3,332)^2}{15} - C_2 = 2,505,373 - 2,501,131 = 4,242$$

Source	D. F.	Sums of squares
Total	8	19,426
Between fertilizers	2	4,242
Between placements	4	10,571
Fertilizers X placements	4	4,613

$$\text{Sum of squares between fertilizer levels (none, 0-20-0, 0-16-8, 4-16-8)} = \frac{(3,661)^2 + (3,616)^2 + (3,332)^2 + (3,729)^2}{15} - C_1 = 3,432,403 - 3,426,304 = 6,099$$

necessary to use a new correction term,  $C_2$ , since only the data from plots actually receiving fertilizers are included in this phase of the analysis. Any variation in the results from plots not receiving fertilizer could certainly not be considered as due to placement. Up to the present time the sum of squares due to treatment has been broken up into some of its various components, namely, fertilizers, placements, and interaction between fertilizer and placements. The remainder of the sum of squares due to treatment is found from the fertilizers and no fertilizer totals. The sum of squares pertaining to this degree of freedom is found at the end of Table 3. By using dummy plots one does not have to give different weights to the totals as each total is found by combining the same number of plots (Table 4). This is the reason why the dummy plots were used.

TABLE 4.—*Procedure for determining the variation between the dummy plots.*

Placement	Blocks					Total
	1	2	3	4	5	
$\frac{1}{2}$ in. none.....	225	227	248	195	197	1,092
2 in. none.....	260	268	283	261	265	1,337
Contact none.....	263	165	266	304	302	1,300
Total.....	748	660	797	760	764	3,729

$$C_1 = \frac{(3,729)^2}{15} = 927,029$$

$$\text{Total sum of squares} = (225)^2 + \dots + (302)^2 - C_1 = 949,121 - 927,029 = 22,092$$

$$\text{Sum of squares between blocks} = \frac{(748)^2 + \dots + (764)^2}{3} - C_1 = 930,536 - 927,029 = 3,507$$

Source	D. F.	Sums of Squares
Total	14	22,092
"Between blocks"	4	3,507
"Within blocks"	10	18,585

The "within blocks" sum of squares may also be found by subtracting the total sum of squares in Table 2 from the total sum of squares in Table 1.

$$\text{"Within blocks" sum of squares} = 72,160 - 53,575 = 18,585.$$

So far the following variances have been segregated, namely, the variance due to blocks, placements, fertilizer treatments, fertilizer levels, interaction between fertilizers and placements, and the error variance. However, if the variance for fertilizers pertaining to stands is compared with the error variance, no significant "F" value results. The same situation is true with regard to the interaction of fertilizers and placements. However, from an examination of the data and from field observations, it was quite apparent that there was a significant difference between fertilizers and also in the interaction between

fertilizers and placements. In Table 3 it appears the difference in stand is the result of the injury to stand caused by the 4-16-8 fertilizer applied in contact with the seed (Fig. 2). This observation, then, points to the fact that perhaps there is a significant difference between fertilizers and also in the interaction between fertilizers and placements. It is necessary to separate further the sums of squares due to fertilizers and the interaction between fertilizers and placements into their respective components.

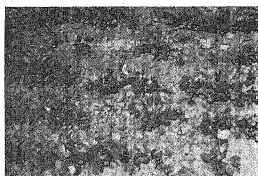


FIG. 2.—Stand of cannery peas on a plot receiving 300 pounds of a 4-16-8 fertilizer applied in contact with the seed.

Since the requirements of orthogonality are satisfied in this design, it is possible to break down the treatment sum of squares. It is possible to do this in a number of different ways and it is up to the investigator to select the way that best suits his purpose. In fact it is highly desirable that this premise be considered at the time the experiment is being designed. In Table 5 the sum of squares due to fertilizers is separated in the manner indicated, namely, fertilizer vs. no fertilizer; 4-16-8 vs. 0-16-8 and 0-20-0; and 0-16-8 vs. 0-20-0. This is accomplished by considering the three comparisons  $[3(0)-(0-20-0)-(0-16-8)-(4-16-8)]$ ;  $[2(4-16-8)-(0-16-8)-(0-20-0)]$ ; and  $[(0-20-0)-(0-16-8)]$ . The scheme for separating the three degrees of freedom is indicated in Table 5.

TABLE 5.—*Procedure for breaking down the treatment variance due to the effect of fertilizer into its components.*

Comparisons					
None	0-20-0	0-16-8	4-16-8	D.F.	
+3	-1 -1 +1	-1 -1 -1	-1 +2	1 1 1	Fertilizer vs no fertilizer 4-16-8 vs (0-16-8) and (0-20-0) (0-20-0) vs (0-16-8)

Sum of squares due to no fertilizer vs fertilizer is

$$\begin{aligned} & \text{none} \quad \text{PK} \quad \text{P} \quad \text{NPK} \\ & [3(0) - (0-16-8) - (0-20-0) - (4-16-8)]^2 \\ & \quad (9+1+1+1) (3) (5) \\ & = \frac{(11,187 - 10,609)^2}{180} = \frac{334,084}{180} = 1,856 \end{aligned}$$

Sum of squares due to (4-16-8) vs (0-16-8) & (0-20-0) is:

$$\begin{aligned} & \text{NPK} \quad \text{PK} \quad \text{P} \\ & [2(4-16-8) - (0-16-8) - (0-20-0)]^2 = (6,664 - 7,277)^2 \\ & \quad (4+1+1) (3) (5) \quad \quad \quad 90 \\ & = \frac{334,084}{90} = 3,723 \end{aligned}$$

Sum of squares due 0-16-0 vs 0-20-0 is:

$$\begin{aligned} & \text{P} \quad \text{PK} \\ & [(0-20-0) - (0-16-8)]^2 = (3,661 - 3,616)^2 = 68 \\ & \quad (1+1) (3) (5) \quad \quad \quad 30 \quad \text{Total } 6,099 \end{aligned}$$



Briefly, the procedure is as follows: In the comparison of no fertilizer vs. fertilizers, the stand from the unfertilized plots in each block is multiplied by 3 and the stands for the remaining treatments in each block are totaled and then the difference existing between the two totals, 11,187 and 10,609, is squared and divided by the proper denominator. This figure represents the sum of squares due to no fertilizer vs. fertilizer. It is important to calculate the proper denominator in order to arrive at the correct sum of squares. In the calculation of this denominator the coefficients in the comparison  $[3(0)-(0-16-8)-(0-20-0)-(4-16-8)]$  are 3, 1, 1, and 1. These coefficients are squared individually and added, resulting in a total of 12. It is then necessary to multiply by 3 since there are three items in each treatment for each replication and again by 5 since there are five replications. Similarly, the sums of squares for 4-16-8 vs. (0-16-8) and (0-20-0) and (0-16-8) vs. (0-20-0) are calculated. A detailed procedure is given in Table 5.

In breaking down the sum of squares for the interaction placement and fertilizers, a different procedure is necessary. This procedure is presented in detail in Table 6.

The complete analysis of variance for stand is recorded in Table 7. The number of plants required for significance is calculated both for the purpose of comparing any two treatment means and also for testing differences between any treatment mean and the mean stand of plots receiving no treatment.

Since the calculation of the data for yield is the same as for stand, only the final analysis of variance for yield is recorded. The analysis for the yield data is presented in Tables 8 and 9.

#### INTERPRETATION AND DISCUSSION OF RESULTS

The data for stand indicate that a significant injury to stand will result if the fertilizer 4-16-8 is placed in contact with the seed at the rate of 300 pounds per acre under soil and weather conditions as they existed in Michigan in 1940.

A significant reduction in stand resulted on plots receiving a contact application of 4-16-8 fertilizer when compared to the stands on plots treated with 0-16-8 and 0-20-0 applied in the same manner. The 0-16-8 did not have any greater effect on the stand than did the 0-20-0.

Considering the three fertilizers, only one difference in stand resulted from variable placement, the 4-16-8 applied in contact with the seed significantly reduced the stand below that obtained where the same or other fertilizers were applied in other ways.

The effect of fertilizer placement on stand was significant at the 1% point, the contact placement of 4-16-8 fertilizer significantly reducing the stand.

The analysis of variance for the yield data is presented in Table 8. The following information can be obtained from this table.

A significant increase in yield resulted from the use of each fertilizer.

The yields from plots receiving 4-16-8 were not significantly different than the yields from plots receiving the 0-16-8 fertilizer.

TABLE 6.—Procedure for breaking down the sum of squares due to the interaction of placement and fertilizers into its components.

$PI \times [2(4-16-8) - (0-20-0) - (0-16-8)]$				$PI \times [(0-20-0) - (0-16-8)]$			
	$2(4-16-8)$	$0-20-0$ +	$0-16-8$	Diff.	D. F.		D. F.
$\frac{1}{2}$ in. out	2,352	2,381		-29	-	$\frac{1}{2}$ in. out	-
2 in. out	2,474	2,570		-96	-	2 in. out	-
Contact	1,838	2,326		-488	-	Contact	-
				-613	2		2

$(-29)^2 + (-96)^2 + (-488)^2$	$248,201$	$\frac{8273.7}{30}$		$(71)^2 + (-28)^2 + (2)^2$	$5,829$	$\frac{582.0}{10}$	
$(1+1+4) 5$	$\frac{30}{(-613)^2 = 375,769}$	$\frac{4773.2}{90}$		$(1+1) 5$	$\frac{10}{(45)^2 = 2025}$	$\frac{582.0}{67.5}$	
C. T. =	$\frac{90}{90}$			C. T. =	$\frac{30}{30}$		

$PI \times [2(4-16-8) - (0-20-0) - (0-16-8)] = 8,273.7 - 4,175.2 = 4,098.5$   
 Sum of squares due to placement  $\times$  fertilizers = 515.4 + 4,098.5 = 4,614  
 To further break up the sum of squares due to the interaction  $PI \times [2(4-16-8) - (0-20-0) - (0-16-8)]$  into sums of squares due to interactions of placements  $\times$  contact (4-16-8)  $\times$  all other treatments and placement  $\times$  difference of 2 (4-16-8) and (0-20-0)  $\times$  (0-16-8) for 2 in. out and  $\frac{1}{2}$  in. out set up the following:

$$\left\{ \begin{array}{l} \text{NPK} \\ \text{contact} \end{array} \right\} \frac{2 \text{ in.}}{2} \left[ \frac{NPK}{PK} \cdot \frac{P}{PK} - \frac{P}{PK} \right] - \left[ \frac{NPK}{PK} \cdot \frac{P}{PK} - \frac{P}{PK} \right] - \left[ \frac{NPK}{PK} \cdot \frac{P}{PK} - \frac{P}{PK} \right] + \frac{2 \text{ in.}}{(10+4+4)} + \frac{(4+1+1)}{(4+1+1)}$$

$$\left\{ \begin{array}{l} \text{NPK} \\ \text{contact} \end{array} \right\} \frac{2 \text{ in.}}{2} \left[ \frac{NPK}{PK} \cdot \frac{P}{PK} - \frac{P}{PK} \right] - \left[ \frac{NPK}{PK} \cdot \frac{P}{PK} - \frac{P}{PK} \right] - \left[ \frac{NPK}{PK} \cdot \frac{P}{PK} - \frac{P}{PK} \right] + \frac{2 \text{ in.}}{(10+4+4)} + \frac{(4+1+1)}{(4+1+1)}$$

$$\left\{ \begin{array}{l} \text{NPK} \\ \text{contact} \end{array} \right\} \frac{2 \text{ in.}}{2} \left[ \frac{NPK}{PK} \cdot \frac{P}{PK} - \frac{P}{PK} \right] - \left[ \frac{NPK}{PK} \cdot \frac{P}{PK} - \frac{P}{PK} \right] - \left[ \frac{NPK}{PK} \cdot \frac{P}{PK} - \frac{P}{PK} \right] + \frac{2 \text{ in.}}{(10+4+4)} + \frac{(4+1+1)}{(4+1+1)}$$

$$4,023 + 75 = 4,098 \text{ (as a check.)}$$

The yields from plots receiving 0-16-8 fertilizer were significantly greater than the yields from plots receiving the 0-20-0 fertilizer.

Fertilizers placed in contact with the seed caused a significant reduction in yield while no difference between yields occurred when either of the side placements were used.

The 4-16-8 fertilizer when applied in contact with the seed gave a significantly greater reduction in yield than did either the 0-16-8 or the 0-20-0 analysis.

TABLE 7.—Analysis of variance showing sums of squares for individual treatment degrees of freedom (stand data).

Source	D. F.	S. S.	Mean square
Total	59	72,160	—
Blocks	4	5,228	1,307
None vs. fertilizer	1	1,856	1,856
NPK vs. P+PK	1	4,175	4,175*
PK vs. P	1	68	68
Total fertilizer	3	6,099†	2,033
Placements	2	10,571	5,286†
Placements×fertilizer (1)	1	4,023	4,023*
Placements×fertilizer (2)	1	75	75
Placements×fertilizers (3)	2	515	258
Total placements×fertilizers	4	4,613‡	1,154
"Within blocks"	10	18,585	—
Treatments×blocks	36	27,064	752

\*Significant at 5% point.

†Significant at 1% point.

‡Sum of the numbers in the bracket above.

Pl×Fertilizer (1) = Pl { (NPK Contact) - [(NPK 2 in. out + ½ in. out) + (P+PK contact, 2 in. out and ½ in. out)] }

Pl×Fertilizer (2) = Pl × { [2 (NPK) - (P+PK) 2 in. - (2NPK) - (P+PK) ½ in.] }

Pl×Fertilizer (3) = Pl × (P - PK)

TABLE 8.—Analysis of variance showing sums of squares for individual degrees of freedom (yield data).

Source	D. F.	S. S.	Mean square
Total	59	2,6103	—
Blocks	4	0.2498	0.0624
None vs. fertilizer	1	1.1875	1.1875†
NPK vs. P+PK	1	0.0051	0.0051
PK vs. P	1	0.1527	0.1527†
Total fertilizer	3	1.3453	0.4484†
Placement	2	0.5375	0.2688†
Placements×fertilizer (1)	1	0.0920	0.0920†
Placements×fertilizer (2)	1	0.0004	0.0004
Placements×fertilizer (3)	2	0.0224	0.0112
Total placements×fertilizer	4	0.1148	0.0287†
"Within blocks"	10	0.1206	—
Treatments×blocks	36	0.2385	0.0066

\*Significant at 5% point.

†Significant at 1% point.

Pl×Fertilizer (1) = Pl × [(4-16-8 contact) - all other treatments]

Pl×Fertilizer (2) = Pl { × [(4-16-8) - (0-16-8 + 0-20-0) for 2 in. - [(4-16-8) - (0-20-0 + 0-16-8) for ½ in.] }

Pl×Fertilizer (3) = Pl × (0-16-8 - 0-20-0)

In regard to the use of a value referring to the difference required for significance between treatment means, a word of caution should be introduced. Although this procedure and interpretation is widely used by investigators in evaluating treatments, it should be realized that it is at best but an approximation and should be treated as such. The significance of the invalidity of the use of such a value is quite apparent since, if 100 samples from a uniformly treated area are taken, 5 out of the 100, on the average, would give values significantly different. Therefore, one should not place too much confidence in a number "required for significance" but should only use this number as an indication of the trend of the results. In other words, it is still impossible to summarize the results of an experiment with one value only.

TABLE 9.—Mean yields of treatments.

	Treatment	Yield, tons per acre
1/2 in.....	0-20-0	0.89
1/2 in.....	0-16-8	1.00
1/2 in.....	4-16-8	1.03
2 in.....	0-20-0	0.77
2 in.....	0-16-8	0.99
2 in.....	4-16-8	0.98
Contact.....	0-20-0	0.70
Contact.....	0-16-8	0.80
Contact.....	4-16-8	0.64
None.....		0.54

Diff. required for significance:

Between any two treatment means = .104.

Between any treatment mean and mean of plots receiving no fertilizer = .085.

### CONCLUSIONS

A detailed procedure for the analysis of variance involving the use of dummy plots is presented. The results from this experiment point out the necessity of separating the sum of squares for treatments into their respective components in order to arrive at a full and correct interpretation of the data.

The necessity of the need for caution in using a value assigned as a difference required for significance between treatments is brought out.


The placement of fertilizers is a very important factor in the culture of cannery peas.

A complete fertilizer placed in contact with the seed at the rate of 300 pounds per acre on a Brookston loam significantly reduced the stand and yield of cannery peas while only the yield was reduced when an 0-20-0 or an 0-16-8 were used. The stand data do not bring out all of the injury actually due to the contact placement of fertilizer since all plants that emerged were included in the stand counts. In plots receiving 4-16-8 and 0-16-8 and to a lesser extent 0-20-0 in contact with the seed, a large number of plants were permanently injured so that this method of placement of fertilizers at the rate used should not be recommended.

The plots receiving the 0-16-8 analysis gave as good yields as did

those receiving 4-16-8 and significantly larger yields than plots on which 0-20-0 was applied.

There was no significant difference in yields from plots on which the fertilizer was applied in bands  $\frac{1}{2}$  inch to the side and  $1\frac{1}{2}$  inches below the seed and plots in which the fertilizer was placed 2 inches to the side and  $1\frac{1}{2}$  inches below the seed.



## A CHEMICAL STUDY OF QUICK-TEST TECHNICS FOR POTASSIUM AND CALCIUM<sup>1</sup>

S. W. MELSTED<sup>2</sup>

THE use of quick chemical tests as an aid in determining fertilizer needs of a soil is today an accepted procedure. The expressions "quick-tests" and "rapid soil tests" are generally accepted as referring to semi-quantitative colorimetric or turbidimetric tests made on a single soil extract. That many of these quick-tests fall short of their expected goal is not surprising when one considers the variety of procedures proposed by various workers. The details of these various methods, as well as comparisons of their practical value, are reviewed by Anderson and Noble (1),<sup>3</sup> Reed and Sturgis (9), Bray (3), Goss and Owens (5), and others. Although attempts have been made to correlate the results of these methods with field responses, few attempts have been made to correlate them with the more accurate laboratory methods of soil analysis.

In general, all quick tests for replaceable bases can be classified into three groups on the basis of the extracting solutions used as follows: First, the weak salt acid extracting solution as used by Morgan (8), Hester (6), Spurway (11), Merkle (7), and others; second, the strong mineral acid extracting solutions as used by Bayer and Bruner (2); and third, the strong neutral salt solution as used by Bray (4). As it was not feasible to make a detailed study of all of the quick tests in use today, Morgan's 10% sodium acetate in 3% acetic acid was chosen to represent the first group, Bayer and Bruner's 0.3 N hydrochloric acid for the second group, and Bray's 22% sodium perchlorate solution as a representative of the third group. Two other extracting solutions, Merkle's 0.25 N sodium acetate and an acid sodium perchlorate solution were also considered to illustrate points in technic.

It is the purpose of this paper to evaluate the technics from a chemical point of view rather than to compare the usefulness of these methods in any specific region. Therefore, all results reported in this paper are based on the quantitatively determined amounts of the ions removed by the extracting solutions used and are not based on any suggested calibrations for field use.

### SOIL EXTRACTIONS

It is obvious that the accuracy of any quick test depends in the first place on the quantitateness with which the extracting solution removes the form of nutrient concerned from the soil. It has been shown that in the case of the cations K, Ca, and Mg plants can use that which is held in the replaceable form as well as that present in

<sup>1</sup>Contribution from the Department of Agronomy, Illinois Agricultural Experiment Station, Urbana, Ill. Presented before the Committee on Fertilizers of the American Society of Agronomy, Chicago, Ill., December 3, 1940. Published with the approval of the Director of the Experiment Station. Received for publication November 22, 1941.

<sup>2</sup>Assistant in Soil Survey Analysis.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 543.

the soil water. Therefore, a quick-test method to be of value must measure either all or a constant predictable proportion of the replaceable cations present.

Goss and Owens (5), Bray (3), and others have investigated extraction technics and reached the conclusion that shaking the soil and extracting solution together before filtering gives results which agree more closely with quantitative results than those secured by leaching the soil in a filter-paper cone. The fact was recognized that when a soil is placed on a filter-paper cone and a solution poured over it, the leaching occurs in a manner such that the first portion of the solution coming through will contain most of the replaceable bases and the last portion relatively little. This produces a plus error which favors the weak extracting solutions unless thorough leaching with a large excess of solution is carried out. This method of extracting was exaggerated to the point where the solution was added drop by drop from a pipette and allowed to leach through the soil. Both Morgan's universal extracting solution, hereafter called universal extracting solution, and Bray's 22% sodium perchlorate solution, which will be referred to as the neutral G-3 extracting solution, were used in a comparison of the leaching method and the shaking method.

Samples<sup>4</sup> of known exchange capacity and exchangeable base content were used, and the percentage of the total replaceable potassium removed was taken as a criterion of the effectiveness of the technics involved. Soils of varying exchange capacity were leached by both solutions using both technics. In all cases the potassium in the extract was determined by the Bray (4) precipitation technic. Fig. 1 shows the data secured.

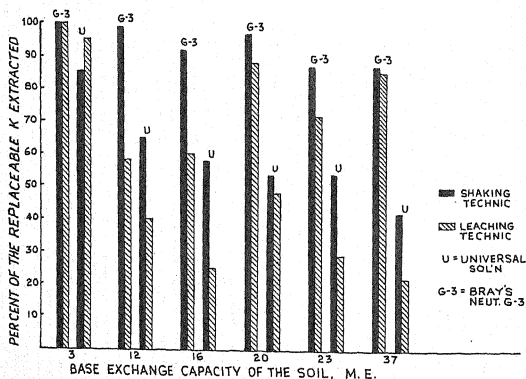


FIG. 1.—A comparison of extracting technics using two extraction solutions.

<sup>4</sup>The author is indebted to the Agronomy Department, University of Illinois, for the detailed analytical data on the soils used in this study.

For coarse-textured soils low in exchange capacity (3 M.E.), the leaching technic with the universal extracting solution gave results that agreed more closely with quantitative results than did those secured with the shaking technic. This better agreement with soils low in exchange capacity is due to the leaching technic. On coarse-textured soils, most of the exchangeable potassium is extracted in the first few milliliters of leachate, leaving the soil saturated with a solution containing less potassium per unit volume than the filtrate. Thus, the filtrate tested will give high results because all quick-test methods are calibrated on the assumption that the potassium is uniformly distributed throughout the total amount of extracting solution used before filtering, and therefore, that the volume of filtrate leaching through the soil may be ignored. With heavier soils of higher base exchange capacity the results obtained by the leaching technic with either solutions were very erratic, probably due to the unequal wetting or lack of uniform penetration of the solutions into the soil. This would be true for any extracting solution. The shaking technic, where the soil and solution were shaken 1 minute before filtering, gave uniform results and was, therefore, used in all subsequent work.

#### EXTRACTION EFFICIENCY OF DIFFERENT SOLUTIONS

*Potassium extraction.*—Fig. 1 illustrates the decreasing ability of the universal extracting solution to remove replaceable potassium from soils with increasing base-exchange capacities. To study the extracting efficiency of various solutions, soils varying in exchange capacity as well as replaceable potassium were used. The amount of potassium removed as compared to the amount of replaceable potassium present, as determined quantitatively, was taken as an index of the extracting ability of the solution.

In all cases, 5 grams of air-dry soil were weighed out, placed in a large glass vial, and 10 ml of the extracting solution were added. The soil and solution were shaken for 1 minute, filtered, and the potassium determined by the Bray precipitation technic. The results are given in Table 1.

The acid G-3 solution is Bray's neutral G-3 solution containing 1%  $\text{HClO}_4$  making the solution approximately 0.1 N as to acid and about 1.8 N as to  $\text{NaClO}_4$ . This solution is included here as it represents the modification of the neutral G-3 solution necessary for complete removal of replaceable calcium, and results with it show the error introduced into the potassium test by such a modification. With the exception of sample No. S6758, the neutral G-3 solution *may be said to remove all of the replaceable potassium, regardless of the exchange capacity of the soil.* The percentage of the replaceable potassium removed by the universal extracting solution decreased steadily as the exchange capacity of the soil increased, varying from about 94% to less than 30% of the amount present. Thus, this solution *cannot be said to remove a constant proportion of the replaceable potassium.* This inability is probably due to the fact that the solution is too dilute to force practically all of the replaceable potassium into solution when an equilibrium between the solution and soil is reached. The 0.3 N



HCl tended to be erratic in its removal of replaceable potassium from soils low to high in exchange capacity. The acid G-3 solution removed amounts greater than the replaceable potassium in almost all cases.

TABLE I.—*A comparison of amounts of potassium extracted by various extracting solutions with the amounts present as determined by laboratory methods.*

Sample No.	Quantitative analysis		Quick-tests							
	Exch. cap., M.E.	Exch. K, lbs. per acre	Neutral G-3 soln.		Universal soln.		Acid G-3 soln.		0.3 N HCl soln.	
			Lbs. per acre	%	Lbs. per acre	%	Lbs. per acre	%	Lbs. per acre	%
S6758	37.0	285	200	70	80	28	270	95	150	53
S7531	37.0	212	213	100	116	55	250	118	213	100
S10553	23.0	195	184	94	116	60	230	118	184	94
S6881	23.0	375	330	88	184	49	330	88	230	62
S6989	20.0	108	111	103	63	57	168	155	150	138
S6866	20.0	145	132	91	75	52	184	127	—	—
S6769	16.0	200	184	92	116	58	213	107	136	68
S6764	11.4	190	184	97	120	63	213	112	176	93
S6765	10.9	157	168	101	105	67	200	127	—	—
S10100	3.5	64	60	94	60	94	96	150	80	125
S10082	3.0	131	132	100	105	80	160	122	120	92
S5153	3.0	62	63	101	60	97	105	170	85	137

\*Percentage recovery of the total replaceable K.

Thus, when weak salt-acid, strong acid, or strong salt-acid solutions are used in the concentrations recommended in published quick-test methods for the extraction of potassium, the results secured will not agree with the quantitative results secured with exhaustive ammonium acetate leaching for soils having a wide range in base-exchange capacities. It would seem, therefore, that a *strong neutral salt extracting solution is preferable to acid solutions for replaceable potassium determinations.*

When acid extracting solutions such as 0.3 N HCl and acid G-3 are used, an error is apt to appear due to the large amounts of aluminum extracted. If aluminum is present in sufficient quantities to interfere with the potash test, the precipitate will appear fluffy and gelatinous on standing rather than granular. Just how high a concentration of aluminum is required before it will interfere with the potash test has not been determined, but it probably is approximately 200 p.p.m. In these determinations the gelatinous type of precipitate was not observed in the case of the acid G-3 solution but was quite marked for the 0.3 N HCl solution. Thus, the higher results obtained with these acid-extracting solutions may be due to the strong displacing power of the hydrogen ion, to an increased solubility of nonreplace-

able potassium compounds in acid solutions, to an error introduced into the potassium determination by the large amounts of aluminum extracted, or to a combination of these factors.

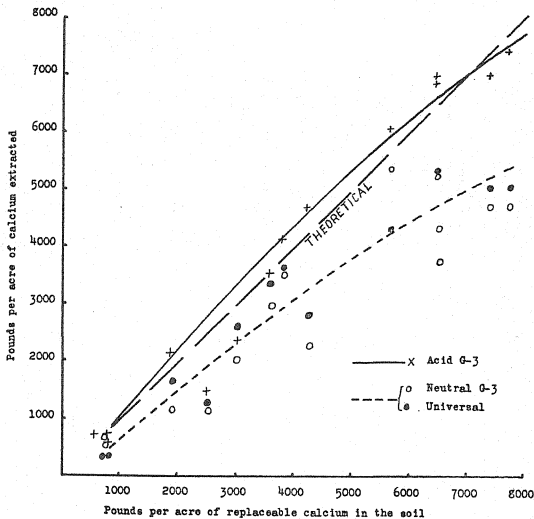


FIG 2.—Calcium extraction with various solutions.

*Calcium extraction.*—The relative extracting power of three of these solutions for calcium is shown in Fig. 2. In all cases 2 ml of saturated ammonium oxalate was added to 2 ml of filtrate, the mixture allowed to stand 15 seconds, and then shaken. The turbidity was read after 2 minutes more and compared with standard solutions. It will be observed that neither the universal nor the neutral G-3 extracting solutions were efficient in extracting calcium. Their average efficiency for all samples was about 75%. It is interesting to note that the amount of calcium present and the exchange capacity of the soil seemed to have little effect on the percentage of calcium extracted by these solutions, although both solutions gave results that tend to deviate downward from the quantitative values as the amount of calcium increases. The acid G-3 extracted amounts of calcium which agreed well with the quantitative results.

## ANALYSIS OF EXTRACT

## ESTIMATION OF K IN EXTRACTS

With one or two exceptions, all quick tests for the determination of potassium in the extract have two things in common, *viz.*, (a) the precipitation of the potassium as sodium-potassium-cobaltinitrite, and (b) the estimation of the resulting amount of yellow precipitate formed. In this study the precipitation technics suggested by Morgan (8), Bray (4), and Baver and Bruner (2) were considered because they represent the changes in technic necessitated by the use of different extracting solutions. Standard solutions made up in the various extracting solutions were used. All reagents were freshly prepared according to directions and the suggested technics carefully followed. After the precipitate was formed, the volume of precipitate suspension required to obscure the standard reading lines, over a constant light source, was measured and recorded.

The Morgan precipitation technic is similar to that of Bray except that much smaller amounts of extract, reagent, and alcohol are used. With standard solutions it was found that both methods gave very excellent results after the mixing technic was learned. It is of the greatest importance that the alcohol be added slowly, forming a double layer, followed by gradual rotary shaking, at first slowly and then more vigorously. The reproducibility of potassium results with the Bray technic, which also represents the accuracy of the Morgan technic, is shown in Table 2. If the shaking is too rapid, the results secured will be too low.

TABLE 2.—*Reproducibility of potassium results using standard solutions and the Bray precipitation technic.*

Trial No.	Standard K solutions in G-3			
	18.8 p.p.m.	23.8 p.p.m.	45.5 p.p.m.	60.0 p.p.m.
1.....	3.5*	3.10*	1.60*	1.20*
2.....	3.6	3.00	1.60	1.25
3.....	3.5	3.00	1.60	1.20
4.....	3.5	3.05	1.60	1.20
5.....	3.6	3.00	1.60	1.10

\*Milliliters of developed precipitate required to obscure the standard reading lines.

Baver and Bruner (2) have developed a good technic for the precipitation of potassium in a dilute HCl solution. Two factors have been considered in developing the technic. First, they have increased the ratio of alcohol to extract (3.3 ml alcohol to 7 drops of extract) which has the effect of increasing the amount of precipitate formed in proportion to the amount of potassium present. Second, they have buffered their alcohol by adding 3 grams of sodium acetate in 100 ml of 95% ethyl alcohol to overcome the effect of the acidity.

While the increased alcohol:extract ratio and buffered alcoholic solution tend to offset the acid error, the buffered alcohol itself introduces an error that is often overlooked. The greater the amount of a

buffering salt in the alcohol, the greater is the decrease in the efficiency of the alcohol to produce a precipitate. The effects of buffering were clearly shown by taking standard solutions of potassium in acid G-3 solution and following the Bray precipitation technic, but using alcohol containing 2 and 5% sodium acetate. Table 3 shows the decrease in the amount of precipitate formed with increasing amounts of buffer. Bayer and Bruner's precipitation technic involving standard solutions of potassium in 0.3 N HCl was used with unbuffered ethyl alcohol and their suggested 3% buffered alcohol. The same relative decrease in amount of precipitate formed is noticed (Table 3). It is suggested that more accurate results might be obtained by neutralizing the filtered test solution with dilute NaOH, or buffering with a few crystals of sodium acetate before the determination is made.

TABLE 3.—*Effect of buffering the alcohol on the amount of potassium precipitate formed.*

Bray precipitation technic, 50 p.p.m. K in acid G-3			Bayer and Bruner technic 200 p.p.m. K in 0.3 N HCl	
Methyl- isopropyl alcohol mixture	2 grams NaC <sub>2</sub> H <sub>3</sub> O <sub>2</sub> in 100 ml methyl- isopropyl alcohol	5 grams NaC <sub>2</sub> H <sub>3</sub> O <sub>2</sub> in 100 ml methyl- isopropyl alcohol	95% ethyl alcohol	3 grams NaC <sub>2</sub> H <sub>3</sub> O <sub>2</sub> in 100 ml ethyl alcohol
1.50*	2.00*	4.00*	0.7*	2.1*
1.60	2.00	4.00	0.8	2.2
1.60	1.95	4.00	0.8	2.0
1.65	1.95	4.00	—	—

\*Ml of developed precipitate required to obscure the standard reading lines.

Many quick-test technics involve the measurements of reagents and extract in drops. Inherently this technic is subject to considerable error due to variations in drop size with various pipettes. Accuracy of results will be increased with any procedure if the extract and reagents are measured in terms of milliliters.

In many suggested technics the developed precipitate is read with an indirect source of light. Such reading technics are liable to large errors due to differences in light intensity and distortion of the reading lines by the glass vials, the latter effect increasing with the distance of the vial from the standard reading lines. A standard light source shining up through the bottom of the vial, and with outside sources of light excluded, should always be used to insure maximum accuracy in estimating the amount of precipitate formed.

The ratio of extract to alcohol in the precipitation of potassium is important. As this ratio widens and more alcohol is used the amount of precipitate formed in proportion to the amount of potassium present increases. However, this tendency may be carried too far resulting in a decrease in the accuracy of the tests. To illustrate the point, if one takes Bayer and Bruner's precipitation reagents with the exception of using unbuffered ethyl alcohol, a slight precipitate is obtained

with the reagents themselves. This is not necessarily a criticism against the Bayer and Bruner technic because with their buffered alcohol this sodium cobaltinitrite precipitate will not form. It suggests, however, that the amount of alcohol used may be very near the critical point, and that poor technic in the use of this test could cause a considerable error.

Whenever potassium is being determined by any of the quick-test methods the usual precautions of controlling the temperature of the reagents and test solution should be followed. In all the work reported in this paper, the temperature of reagents and extract during precipitation was maintained between 22° and 25° C.

#### CALCIUM PRECIPITATION TECHNICS

Many investigators report excellent agreement between determinations by quantitative laboratory methods and the rapid tests for calcium. However, it has been the author's experience that most of the suggested quick tests for calcium are less accurate than those for potassium. Two reasons for this low accuracy are presented. First, with the possible exception of the acid G-3 extracting solution, most of the extracting solutions do not quantitatively extract the replaceable calcium from the soil; and second, as reported by Snell (10), the turbidimetric determination of calcium as oxalate has an experimental error of approximately 20%.

The precipitation technic used in this study to determine calcium was essentially as follows: 2 ml of a standard calcium solution was measured into a flat-bottom vial and 2 ml of saturated ammonium oxalate added, allowed to stand 15 seconds without shaking, then shaken and the turbidity estimated after 2 minutes. A standard light source was used in estimating the amount of precipitate formed.

The accuracy of the calcium precipitation technic was investigated by using standard solutions of calcium in the G-3 solution. The reproducibility of calcium results with standard solutions is presented in Table 4. The method is most accurate for concentrations between 150 and 350 p.p.m. Above this concentration it becomes difficult to distinguish between successive increases in calcium concentrations. It will also be noticed that the method shows about a 12½% error. This may be considered as a relatively good agreement for a turbidimetric determination of calcium.

The time lapse between the addition of the ammonium oxalate and the shaking of the mixture had a considerable effect on the turbidity produced. The time factor appears to be most important in the lower concentrations. In the higher concentrations the precipitate forms so rapidly that the time factor becomes less important. As the results secured for the 15-second and the 20-second intervals were comparable, that approximate interval was chosen for all the work in this paper.

Stabilizing agents such as alcohol, to form a fine-textured precipitate, or glycerol, to increase the viscosity of the solution, are often used to prevent the calcium precipitate from settling out too rapidly for turbidimetric readings. While some success was secured with alcohol, the use of glycerol decreased the accuracy of the test markedly.

TABLE 4.—*Reproducibility of calcium turbidity readings with standard solutions.*

No. of trial	Standard solutions of calcium								
	100 p.p.m.	150 p.p.m.	200 p.p.m.	250 p.p.m.	300 p.p.m.	350 p.p.m.	400 p.p.m.	450 p.p.m.	500 p.p.m.
1	3.15*	3.00*	2.40*	2.10*	1.50*	1.10*	0.90*	0.90*	0.80*
2	3.20	3.00	2.45	2.15	1.55	1.15	0.90	0.85	0.80
3	3.25	3.05	2.40	2.20	1.65	1.10	0.85	0.90	0.70
4	3.35	3.00	2.60	2.15	1.60	1.05	0.90	0.85	0.85
5	3.30	3.10	2.60	2.15	1.50	1.10	0.90	0.80	0.80
6	3.20	3.00	2.50	2.10	1.60	1.05	0.90	0.85	0.80
7	3.20	3.10	2.40	2.05	1.50	—	—	—	—
8	3.40	3.00	3.00	2.15	1.55	—	—	—	—
9	3.20	3.00	2.50	2.20	1.40	—	—	—	—
10	3.25	2.90	2.50	2.20	1.60	—	—	—	—
11	3.40	2.85	3.00	2.15	1.40	—	—	—	—
12	3.40	3.05	2.50	2.00	1.50	—	—	—	—

\*Ml of developed precipitate required to obscure the standard reading lines.

Some quick-test methods for calcium suggest that the precipitation of the calcium by sodium oxalate be done at a pH value of 8 rather than at the lower pH levels suggested by quantitative procedures for the separation of calcium from magnesium. While this phase has not been carefully studied, tests thus far made indicate that such an alkaline precipitation method should be carefully studied from the standpoint of sesquioxide interference and the quantitative separation of calcium from magnesium before being recommended for general use.

#### DISCUSSION

The principal factor which determines the accuracy of any quick-test for replaceable bases is the quantitateness with which the extracting solution removes these bases from the soil. Therefore, the first consideration in the selection of a quick-test method must be the extracting solution. The data presented here indicate that there is no one extracting solution in use for rapid tests that is quantitative for all of the replaceable bases. It appears that many of the extracting solutions recommended today are but a compromise between the extremes in inaccuracy which would result if they were made quantitative for one base and the other bases disregarded. It is the author's judgment, based on the data presented, that the use of the single extracting solution now in general use for all nutrients should be discouraged in favor of more accurate methods.

After choosing an extracting solution that will quantitatively extract a particular nutrient from the soil, the accuracy of its determination becomes important. Before any method is adopted it should be thoroughly studied and its accuracy tested with standard solutions. There is no justification for the use of a method or technic in testing a soil extract that does not give satisfactory results with standard solutions. The thought seems to prevail that the method or technic used need only distinguish between large differences in the

amounts of that nutrient present in the soil extract. The contrary is true. One cannot hope to be able to interpret small or large differences unless the results are known to be accurate. When quantitatively accurate quick tests are attained their intelligent interpretation will follow.

Considerable effort has been made in attempting to correlate the methods of reporting quick-test results. The confusion in the interpretation and meaning of reported results, i.e., high, low, or medium, lies in the fact that these results are not quantitative and too often bear no constant relation to the amounts present in the soil. Rather than attempt to correlate various quick-test results that have no definite relation to each other or to the amount present in the soil, it is suggested that the amount held in the exchangeable form, as determined by quantitative methods, be used as a basis for comparison. Thus, for the exchangeable bases, we would have an acceptable standard with which to compare quick-test results. Quick tests should be made as nearly quantitative as possible and the results reported as M.E. per 100 grams, pounds an acre, or p.p.m. in the soil.

#### SUMMARY

A study of the quantitative extraction of replaceable potassium and calcium from soils by several extracting solutions was made. Technics used in the determination of these bases in the extracts were evaluated. The following conclusions are based on the data presented:

1. The accuracy of any quick test for replaceable bases depends primarily on the quantitateness with which the extracting solution removes these bases from the soil.
2. None of the extracting solutions recommended for the simultaneous extraction of all the nutrient ions has been found to extract all of the exchangeable bases quantitatively.
3. Any quick-test method that measures the aliquot of extract in drops is subject to errors.
4. A turbidimetric or colorimetric method that does not give accurate results with standard solutions cannot be expected to work satisfactorily for quick tests.
5. The determination of potassium in a filtrate by methods involving modifications of the cobaltinitrite method may show variations caused by (a) the order of adding the reagents, (b) the temperature at which the precipitation is made, (c) the mixing technic involved, (d) interference by other ions, (e) the ratio of alcohol to solution, and (f) the method of estimating the amount of precipitate formed.
6. The turbidimetric determination of calcium as the oxalate in the filtrate is subject to errors that may be caused by (a) order of adding reagents, (b) the time interval between the adding of reagents and shaking, (c) the amount of calcium present, (d) the use of stabilizing agents, (e) the pH at which the precipitation is made, (f) method of estimating the amount of precipitate formed, and (g) presence of interfering ions.

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ROW WIDTHS AND COTTON PRODUCTION<sup>1</sup>D. M. SIMPSON AND E. N. DUNCAN<sup>2</sup>

DURING recent years, mounting cotton surpluses have caused the adoption of control programs limiting the cotton acreage of individual farms. Soil-conservation programs, with remuneration to farmers for planting soil-improvement crops, have been effective in further reducing cotton acreage. These acreage-reduction programs have tended to increase the intensive use of land and have spurred the farmer into greater effort to increase yield on smaller acreage. In the main, these effects have been beneficial, but certain cultural practices used for the purpose of increasing acre yields may hinder the economical production of cotton.

Less-than-normal distances between rows have been advocated as resulting in higher yields per acre. Such recommendation seemingly is supported by the mass of experimental evidence as currently interpreted; yet, paradoxically, the same data may support a recommendation for wider rows.

Re-examination of the evidence is warranted in view of the technological factors entering into the production of cotton.

## LITERATURE REVIEW

The literature on cotton spacing is extensive, and recommendations have varied from very wide to very close spacing between plants in the row. These contradictory recommendations are concrete evidence of the wide adaptation of the cotton plant and of the varied conditions under which it is grown. Under pre-boll-weevil conditions, Duggar (5)<sup>3</sup> in Alabama in 1886 obtained nearly constant yields with plants spaced 1, 2, or 3 feet apart. Practically identical yields were obtained with plant spacings of 1, 2, 3, and 4 feet in 1887 and in 1889. Fox (6) in Mississippi concluded that on old land, producing about 1 bale per acre, the best yields were obtained with rows 4 feet apart and plants 2 1/2 feet apart in the rows; and that as the land is made richer more distance would be necessary.

The advent of the boll weevil into the eastern section of the Cotton Belt and the need for earlier maturity brought about radical changes in cultural practices. The system of single-stalk cotton culture advocated by Cook (3, 4) aroused new interest and led to a general review of spacing problems. After 1914, practically all investigators agreed that closer spacing than was generally practiced would result in increased yields and earlier maturity and that the cotton plant could adjust itself to a comparatively wide range of spacings.

Mooers (9) in 1928 summarized the data from the various experiment stations over the Cotton Belt and found that the "best" average spacing for the Belt as a whole was between 11 and 16 inches; that if two or more plants per hill were left, the optimum distance was increased; and that the "best" spacing is closer under conditions of high fertility than low fertility.

Ware (12) made comprehensive spacing studies in Arkansas and concluded

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<sup>2</sup>Associate Agronomist and Senior Scientific Aid, respectively.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 552.

that the adaptability of the cotton plant to space is such that yields under favorable growing conditions would not be significantly different, whatever the spacing, within limits of 10,000 to 50,000 plants per acre. Further evidence of the adaptability of the cotton plant to space is shown by Neely (10). In studying the effect of irregular stands on yield, it was determined that the plants at the ends of skips and in sections of rows adjacent to skips yielded considerably more than other plants. In three-row plots, with rows 25 feet in length and 40 inches apart, skips in the center row even up to 10 feet did not lower the plot yield to any appreciable extent.

Several investigators have advanced the idea that yields tend to increase as the space given each plant approaches a square (1, 11), but they have not recommended complete compliance with this theory because very narrow rows are difficult to cultivate properly. Investigations of row width have been made at Mississippi (2), Arkansas (1), South Carolina (7), Alabama (8), and other experiment stations. Almost without exception the results have shown that yield *per acre* increased consistently as width of rows decreased. In general, however, recommendations have not been for extremely narrow rows, as would be indicated by the published results. Hesitancy to recommend a cultural practice indicated as desirable may, be explained by a more careful interpretation of the technological factors entering into cotton production.

#### EXPERIMENTAL BACKGROUND

The results of width-of-row experiments in practically all cases have been reported on the basis of yield *per acre*. If yields are calculated on the basis of yield per linear foot of row, these same figures, almost without exception, will show that yield *per row* increased consistently as width of rows increased. The farmer who grows an acre of cotton in  $2\frac{1}{2}$ -foot rows must fertilize, plant, chop, hoe, plow, and harvest 17,424 linear feet of row. If his rows are  $4\frac{1}{2}$  feet apart, his task is reduced to 9,680 linear feet. As cotton is a row crop, this row footage becomes highly important as a factor in labor, fertilizer, and equipment costs.

Comparisons of yield *per acre* with yield *per work unit* are enlightening. Such comparisons, made by the present authors, for row-width experiments in Mississippi (2) and Arkansas (1) are given in Table 1. These data illustrate the tendency toward increased yields *per acre* with narrow rows and toward increased yields *per row* with wider rows. In the results of the cotton contest in South Carolina, Hamilton and Pritchard (7) report the effects of row width on yield *per acre* and show that the average row width has been reduced materially since the beginning of the contest in 1926. The authors state that the decrease in row width has been carried into general cotton production and no doubt has been a large factor in increasing average yield *per acre* in the state. A comparison of the *per-acre* yields with yield *per work unit* (Table 2) may leave some doubt as to the economy of cotton production in narrow rows.

The results (Table 3) of row width experiments in Alabama (8) indicate that on well-grown cotton, approximately equal *acre* yields were obtained with rows spaced  $2\frac{1}{2}$ ,  $3\frac{1}{2}$ , or  $4\frac{1}{2}$  feet apart. On unfertilized soil the closer rows resulted in higher yields *per acre*, but in

all cases yield per row was materially increased by widening the rows. In the Alabama experiments the number of plants per acre was held constant by inverse variations in the width of rows and drill spacings. This may account for the unusually good results with wide rows in Alabama, as sufficient plants per acre were supplied, even with the wide spaced rows, to provide an adequate framework for a good crop.

TABLE 1.—*Results from row width experiments in Mississippi and Arkansas recorded on the basis of yield of seed cotton per acre in comparison with yields re-calculated by the present authors on the basis of equivalent row lengths.*

Width of row, feet	Mississippi, 1911-15		Arkansas, 1917	
	5-year average		Seed cotton per acre, lbs.	Seed cotton per 17,424 linear feet of row, lbs.
	Seed cotton per acre, lbs.	Seed cotton per 17,424 linear feet of row, lbs.		
3	1,446	1,735	1,107	1,328
3½	1,301	1,821	895	1,253
4	1,161	1,858	913	1,461
4½	1,111	2,000		
5	1,022	2,044	781	1,562
6			584	1,402

TABLE 2.—*Effect of row width on yield, South Carolina cotton contest, 1926 to 1936.*

Width of row, in.	Number of plots	9-year average	
		Lint cotton per acre, lbs.	Lint cotton per 17,424 linear feet of row, lbs.*
30-34	309	616	657
35-37	860	565	678
38-40	886	561	729
41-43	910	552	773
44-46	437	528	792
47-49	352	512	819
50-60	82	489	897

\*Calculated by the authors of this paper.

Under actual farming conditions, the question of profit depends largely upon the efficiency with which the farmer's resources are used. These resources of labor, capital, equipment, and land each play a part in final profit or loss. The amount of labor and equipment expense will vary directly with the number of rows. Cotton is a row crop; it is planted, cultivated, and harvested by the row. Row widths may be varied from 2½ feet to 4½ feet without materially increasing the labor, capital, and equipment costs per row, although in so doing the acreage is increased by 80%. Only the factor of land

has been materially increased. The almost universal practice of reporting yields on a *per acre* basis focuses attention on the most economical use of land and leaves largely out of consideration the economical use of labor and equipment. In the case of row width experiments this may lead to entirely erroneous conclusions. The question of costs and profits from the use of the land is of more concern to the farmer than is the actual per acre yield.

TABLE 3.—Results from row width experiments at two locations in Alabama recorded on the basis of yield of seed cotton per acre in comparison with yields re-calculated by the present authors on the basis of equivalent row lengths.

Width of rows, feet	Main station*				Wiregrass station†			
	Seed cotton per acre, lbs.‡		Seed cotton per 17,424 linear feet of row, lbs.		Seed cotton per acre, lbs.		Seed cotton per 17,424 linear feet of row, lbs.	
	600 lbs. per acre 6-10-4	Unfertilized	600 lbs. per acre 6-10-4	Unfertilized	600 lbs. per acre 6-10-4§	300 lbs. per acre 6-10-4**	600 lbs. per acre 6-10-4	600 lbs. per acre 6-10-4
2½	1,046	683	1,046	683	1,262	1,025	1,262	1,025
3½	1,055	559	1,477	783	1,274	1,047	1,784	1,466
4½	1,026	511	1,847	920	1,248	765	2,246	1,377
5½	977	472	2,149	1,038	1,213	749	2,669	1,648
6½	—	—	—	—	1,141	902	2,966	2,345

\*20,027 plants per acre in all row widths.

†16,000 plants per acre in all row widths.

‡Five-year average.

§Three-year average.

\*\*Two-year average.

If increases in yield per row are obtained with wider rows, the increases should be credited to land use in the same way as increases due to heavier fertilization would be credited to fertilizer. Fertilizer recommendations usually are based on the quality and amount that will give the best chances of profit without undue risks from crop hazards. For instance, largest net increases from the use of fertilizer usually are obtained from the first 300 pounds, as compared with no fertilizer. A smaller but still good profit may be obtained from the second 300 pounds. A third 300 pounds may give a small profit, but the additional cost may be considered too much to risk for the small profit involved. When returns from increased fertilizer applications diminish to a point of low profit, further increases are uneconomical.

The use of additional land for cotton production is analogous to the use of additional fertilizer. Rows may be widened without material increase in cost of production per row, except from the greater use of land. It seems reasonable to assume that land use or row widths should be adjusted to the point at which costs will be the lowest per pound of cotton produced. With present production restrictions based on crop acreage the above principle may not be entirely applicable on farms that do not have alternative uses for farm labor and equipment. On small farms with limited cotton acreage, more in-

tensive use of land may be necessary in order to provide for sufficient volume of production and for efficient use of labor and equipment.

## EXPERIMENTS AT KNOXVILLE, TENN.

### METHOD OF INVESTIGATION

The results of agronomic and cultural tests of field crops are expressed almost universally as a relation between yield and area of harvest. This measure of the efficiency of a particular procedure or method may be misleading unless factors of labor, equipment, and other items of cost are taken into consideration, as well as the customary factor of land area.

The present experiments, begun in 1938 and continued in 1939 and 1940, were designed to test the effect of row width on yield of cotton. All plots contained the same number of linear feet of row. Cost factors of man and animal labor, tool hours, seed, and fertilizer were equal for all plots; thus, the only variable cost factors between plots were breaking and harrowing land prior to planting, picking charges, and land area.

The design of the experiment provided a range of row widths of  $2\frac{1}{2}$ , 3,  $3\frac{1}{2}$ , 4, and  $4\frac{1}{2}$  feet. Six four-row plots of each row width were planted each year. The plots were 100 feet in length. Three plots of each row width were thinned to one plant every 12 inches in the row, and the other three were thinned to two plants every 12 inches. The test plots were randomized so that the data could be analyzed by the variance method. The yields reported herewith are from the two inside rows of each plot, the outside rows being used as guards to minimize lateral competition between adjacent plots of different row widths. In the 1940 test, because of irregularities in soil and moisture, the cotton plants on portions of series 3 were rank in growth and the bolls failed to open before frost. Yields from series 3 were not included in the total yields from the 1940 test.

### EXPERIMENTAL RESULTS

Average plot yields by row width and by plant spacing for the years 1938, 1939, and 1940, are given in Table 4. A combined analysis of these data is shown in Table 5. Yields varied considerably from season to season, but the trend toward increased yields per row from wider rows was similar in all years. Yield per plot (200 linear feet of row) increased in fairly regular steps as the distance between rows increased. These differences, due to row width, were significant in each year and highly significant for the data combined for 3 years. The spacing of the plants in the row, one or two at 12 inches, did not influence yields. In  $2\frac{1}{2}$ -foot rows this would give an approximate variation of from 17,000 to 34,000 plants per acre and is evidence of the wide adaptation of the cotton plant to spacing. There was no significant interaction between years  $\times$  row widths, years  $\times$  plants per hill, row widths  $\times$  plants per hill, or years  $\times$  row widths  $\times$  plants per hill. Significant side comparisons indicate that (a) the differences among years were consistent for the various widths of rows, (b) the differences among years were consistent for number of plants per hill and (c) the differences among row widths were consistent in the various years.

TABLE 4.—*Yields of seed cotton from row width tests, Knoxville, Tenn., 1938, 1939, and 1940.*

Width of row, feet	Plants per hill	Average yield of seed cotton, lbs.						
		Per plot (200 linear feet of row)			By row width and spacing	By row width	By spacing	
		1938	1939	1940			1 plant	2 plants
2½	1	15.4	15.3	24.2	18.3	17.5	22.7	22.4
2½	2	16.7	16.1	17.4	16.7			
3	1	17.1	16.9	28.6	20.9	20.1		
3	2	16.9	17.2	23.8	19.3			
3½	1	18.0	21.4	28.8	22.7	23.5		
3½	2	20.5	21.4	30.8	24.2			
4	1	18.2	23.8	31.1	24.4	24.6		
4	2	20.5	23.1	31.0	24.9			
4½	1	22.4	25.3	33.3	27.0	26.9		
4½	2	20.2	25.1	34.8	26.7			
Diff. for significance at odds of 99:1.....					3.9	2.8	1.7	

TABLE 5.—*Analysis of variance and F values for yield of seed cotton from row width tests, Knoxville, Tenn.*

Variance	D/F	Sum of squares	Mean square	Side-comparisons	F value
Total.....	79	2690.79	—		—
Blocks within years	5	74.81	14.96		
Years.....(Y)	2	1219.66	609.83		72.24*
Width.....(W)	4	802.21	200.55		23.76*
Plants per hill..(P)	1	0.27	0.27		.03
Y X W.....	8	111.81	13.98		1.66
Y X P.....	2	16.92	8.46	(a)	1.00
W X P.....	4	18.78	4.70	(b)	.56
Y X W X P.....	8	66.43	8.30	(c) (d)	.98
Error.....	45	379.90	8.44	(e)	

S. D. = 2.91.

\*Significant at odds of 99:1.

It might be expected that if row widths were increased beyond the maximum distance to which the plants were able to adjust themselves, yield per row would tend to level off. In the present experiments, however, with maximum row widths of 4½ feet, no leveling off in yield is indicated. The upper limit of row width above which yield per row would not increase was not obtained in this experiment.

Of the 802.21 total sum of squares for row width, 785.00 is accounted for by the linear degree of freedom, or by fitting a straight line to row widths. Quadratic, cubic, and quartic degrees of freedom,

or orthogonal polynomials of degrees 2, 3, and 4, with total sum of squares, 17.21, do not contribute a significant amount of variance for row width. These degrees of freedom may be visualized as the amount of variance accounted for by fitting a straight regression line and, successively, curves of the second, third, and fourth degrees to row width means. The separation of total sum of squares for row width into the four component parts shows that the relationship is largely linear and that half-foot increments have substantially the same effect for wide rows as for narrow ones.

### DISCUSSION

If the yield data of Table 4 were reported on the basis of yield of seed cotton per acre, the results would be as shown in Table 6. These figures indicate higher acre yields with closer rows and as thus presented are in accord with the findings reported from Mississippi, Arkansas, South Carolina, and Alabama (Tables 1, 2, and 3). Recommendations based on these data, however, would be highly misleading unless the variable factors of labor and other production costs were included. In the production of a row crop such as cotton, it is apparent that a difference in labor costs must result when row footage per acre is changed. The number of linear feet of row per acre for the different row widths, as shown in Table 6, varies from 9,680 to 17,424.

TABLE 6.—*Yields of seed cotton from row width tests at Knoxville, Tenn., expressed in terms of yield per acre.*

Width of row, feet	Row footage per unit, linear feet	Yield of seed cotton per acre, lbs.			
		1938	1939	1940	3-year av.
2½	17,424	1,398	1,365	1,816	1,526
3	14,520	1,235	1,238	1,900	1,458
3½	12,446	1,199	1,333	1,853	1,462
4	10,890	1,054	1,276	1,691	1,340
4½	9,680	1,031	1,267	1,647	1,315

The efficiency of production methods should be judged by cost per pound or profit per unit of product and not upon yield per acre. A summary of yield and cost data, based on the experiments of 1938, 1939, and 1940, is given in Table 7. In this table the comparative values of the cotton produced with different row widths are based on units of equal linear feet of cotton row. These units vary in size from 1 acre to 1.8 acres, but the production costs per unit are largely the same except for flat breaking and harrowing the additional land, land rent, and picking the additional cotton. In calculating the additional costs for the wider rows, breaking and harrowing the land was figured at \$3.00 per acre, land rent at \$5.00 per acre, and cotton picking at \$0.75 per hundredweight. The value of the seed cotton was placed at 4 cents per pound.

In each of the three years, substantial increases in yield per row foot were obtained with wider rows. The average value of the seed

cotton from units of equal row footage ranged from \$61.04 to \$93.52. After deduction of extra costs incurred with wider rows, an excellent profit is shown for the use of additional land. The limit to which row widths might be increased profitably would not greatly exceed 4½ feet, since with present equipment rows wider than 4½ feet could not be cultivated readily without running additional furrows and increasing the cost of cultivation.

TABLE 7.—*Comparative yields and values of cotton produced on units of approximately equal labor and equipment requirements in row width tests, Knoxville, Tenn., 1938, 1939, and 1940.*

Width of row, feet	Row footage per unit, linear feet	Area per unit, acres	Yield of seed cotton per unit, lbs.			Averages (3-year)			
			1938	1939	1940	Yield per unit, lbs.	Value per unit	In-creased cost per unit*	Com-parative value
2½	17,424	1.0	1,398	1,365	1,816	1,526	\$61.04	—	\$61.04
3	17,424	1.2	1,482	1,485	2,280	1,749	69.96	\$ 3.27	66.69
3½	17,424	1.4	1,679	1,866	2,594	2,046	81.84	7.10	74.74
4	17,424	1.6	1,686	2,042	2,705	2,144	85.76	9.44	76.32
4½	17,424	1.8	1,856	2,195	2,964	2,338	93.52	12.49	81.03

\*Includes additional land rent, tillage, and picking costs.

Acreage increases in soil-depleting row crops and increased production of cotton have been generally discouraged during the past several years. Interpretation of the data from the row width tests at Knoxville on the basis of maintenance of fixed production may present the case in a more popular light. In Table 8, the row footage and acreage of the various row widths necessary to maintain production equivalent to the highest acre yield have been calculated. Yields were maintained from the narrowest to the widest rows with a maximum increase in acreage of only 17%, and at the same time row footage, with attendant costs of fertilizing, planting, and cultivating, was reduced 35%.

TABLE 8.—*Calculated row footage and area to maintain yield equivalent to highest yield per acre from row width tests at Knoxville, Tenn.*

Width of row, feet	Row footage required to produce 1,526 pounds of seed cotton, linear feet	Area of unit, acres
2½	17,424	1.00
3	15,202	1.05
3½	12,996	1.04
4	12,402	1.14
4½	11,373	1.17

The authors do not wish to convey the idea that wider rows will result in more economical cotton production under all circumstances. The results at Knoxville and the data from experiments in Mississippi, Arkansas, South Carolina, and Alabama indicate, however,



that the subject of row widths should be re-examined and the findings interpreted so as to give consideration to all the technological phases of cotton production. Local conditions in respect to land availability, land value, and labor costs are important considerations in determining the profit or loss from intensification of land use.

### SUMMARY

A review of results of row width experiments with cotton indicate that narrow rows yield more *per acre* but wider rows yield more *per row*. As cotton is a row crop and since the operations in connection with planting and cultivating are done by the row, linear feet, or row footage per acre, is of much importance in determining the cost of production. Row widths may be varied from  $2\frac{1}{2}$  to  $4\frac{1}{2}$  feet without materially changing the cost of cultivation per row. The use of additional land with wider rows is analogous to the use of additional fertilizer. In general, row widths should be adjusted to the point at which the costs incurred in production will result in the lowest cost per pound of cotton produced or the greatest production return for labor and equipment.

Experiments at Knoxville, Tenn., over the 3-year period of 1938-40 showed that yields of cotton per row consistently increased as row widths were widened from  $2\frac{1}{2}$ , 3,  $3\frac{1}{2}$ , and 4 to  $4\frac{1}{2}$  feet. The increases in yield were of sufficient size to return an excellent profit for the use of the additional land occupied by the wider rows.

In view of trends toward more intensive land use, the subject of row widths should be re-examined and consideration given to local conditions regarding land value, labor, and equipment costs.

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METHODS OF BREEDING CRESTED WHEATGRASS,  
*AGROPYRON CRISTATUM* (L.) BEAUV.<sup>1</sup>

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CRESTED wheatgrass, *Agropyron cristatum* (L.) Beauv., was studied because of its promise as a drought-resistant, winter-hardy grass for use in western and northwestern Minnesota. The primary objectives were to study the variability among plants tested in individual plant nurseries and in replicated clonal progenies and to determine the effects of self-fertilization in relation to many characters. The characters studied included yield, plant height, damage from root rot, fertility, chromosome number, pollen size, pollen fertility, and content of beta carotene pigment.

Crested wheatgrass is naturally cross-pollinated. Two types have been studied by various workers. The shorter, leafier type was selected at the University of Saskatchewan (9)<sup>3</sup> and given the variety name of Fairway. The taller and more variable type is usually called the forage or standard type. The Fairway variety has given somewhat higher yields of hay than the forage type in trials conducted in Saskatchewan (18). Reitz, *et al.* (15) reported that the Fairway variety yielded slightly less than the forage type in limited tests in Montana.

The forage types appeared much more variable in Minnesota in plant characteristics than the Fairway variety and contained some very vigorous and leafy plants. Because of these facts, the forage type was used principally in these researches on breeding methods.

REVIEW OF LITERATURE

Very little data are available on the value of different methods of breeding the forage grasses. The various breeding methods which are available to breeders of forage grasses have been discussed by many workers. Space permits the review of only those papers which seem to have some relationship with the findings from this study.

Hayes and Clarke (5), in 1925, observed that self-fertilization in timothy did not lead to as great a reduction in vigor as in corn. Some selfed lines yielded less and others considerably more than the average of the commercial variety. Lines were readily obtained which excelled in such important characters as yielding ability and disease resistance. Few abnormalities were observed among the selfed

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 565.

progeny. Clarke (4), in 1927, reported that selection in selfed progenies in timothy developed lines which were vigorous, high yielding, and very uniform for morphological characters and that such selection within self-fertilized lines is an effective and practical means of improving timothy.

Stevenson (17) described an improved strain of brome grass, introduced under the name of Parkland, which was developed from selection within inbred lines for several successive generations. This strain differed from the commercial types in that the plants lacked the vigorous creeping habit and were shorter in plant height and much leafier. The Parkland strain and commercial types yielded about the same. The Parkland strain produced a much heavier aftermath than the commercial and was more drought resistant.

Stevenson (17) in 1937 reported that improvement of crested wheatgrass through selection within inbred lines had been largely abandoned in Canada because of the enormous loss in vigor brought about through inbreeding. Not a single inbred line had been developed which was as desirable from a hay and pasture standpoint as the original material. He stated that improvement in this forage species would be continued essentially by the use of mass selection. Six promising strains developed from selection were being studied further. From these few reviews, it seems that the value of selection within inbred progenies varies with different species of grasses.

Peto (14) reported the  $2n$  chromosome number for the taller and more variable type of crested wheatgrass as 28, and for the shorter more uniform type as 14. Probably the latter type was typical of the Fairway variety which was selected later in Saskatoon, Canada. He observed that the 28-chromosome type showed a high degree of variability. One aneuploid plant with 29 chromosomes was found which was distinctly less vigorous. Myers in a report to Vinall and Hein (19) observed a somatic chromosome number of 14 in the Fairway variety.

Myers and Hill (11, 12) reported recently on the association and behavior of chromosomes in meiosis in the 28-chromosome types of crested wheatgrass. It was described as an autotetraploid. Such conditions, it seems, should give rise to a certain amount of aneuploidy. In a study of six plants, Myers and Hill observed one aneuploid plant which had clearly more than a  $2n$  number of 28 chromosomes. Because of the type of chromosome pairing in the forage type of crested wheatgrass, the inheritance should be of a tetrasomic type. In contrast to this, inheritance in the Fairway type should be of the diploid type.

A number of diseases which attack small grains have been observed also on introduced and native grass species. Although specific mention of the diseases on crested wheatgrass are few, a number of leaf spots, rust, smuts, root rots, and seedling blights have been reported on various species of Agropyron. Christensen (2, 3) reported infection from *Helminthosporium sativum* on crested wheatgrass and other Agropyrons. Padwick and Henry (13) studied the infection of root rot organisms upon species of native grasses. From artificial inoculations, crested wheatgrass was highly susceptible to *Ophiobolus graminis*, moderately susceptible to *Helminthosporium sativum*, and slightly susceptible to *Fusarium graminearum*, although the latter organism did reduce seedling emergence. No reports of differences in disease reaction between the Fairway and forage types were found.

Beta carotene from the leaves of green plants has been shown by many workers to be an important precursor of vitamin A. Atkeson, Peterson, and Aldous (1) found a wide range of variation in carotene content between different species of grasses. Johnson and Miller (8) found that clonal lines of Fairway crested wheatgrass and of Parkland brome grass varied widely in percentage of beta carotene.

It seems from these studies that there are hereditary variations in the beta carotene content of forage grasses, and that the improvement of the nutritive value could be an important part of a breeding program.

### MATERIALS AND METHODS

Two sources of the forage type and one source of the Fairway type of crested wheatgrass were used in these studies. Individual plant nurseries of both types were started at University Farm and studied for two years. The plants in these nurseries were started in the early spring from seed planted in sterilized soil in flats in the greenhouse. The seedlings were transplanted into the field about the first of May but did not make an extensive growth until fall so that the studies on the individual plants were made the second year. The plants were selected on the basis of their plant type and vigor. The selected plants were selfed during the second year in order to obtain inbred progenies and to determine the amount of seed setting when selfed. The selfed progenies were studied later in individual plant nurseries in the same way as the open-pollinated plants.

In the fall of the second year, the plants selected in the individual plant nursery were planted as clonal progenies in replicated trials. One clonal row of a given plant consisted of five plantlets spaced 9 inches apart within the row. The rows were 3 feet apart.

The characters which were studied in all the clonal progenies of the forage type included yield, plant height, and root rot injury. Self-fruitfulness, pollen size and pollen fertility, chromosome number, and beta carotene content were studied with some of these plants which were tested as clonal progenies. Yield was the only character studied in the Fairway clonal progenies.

Yields are given in grams on a dry matter basis for the forage type selections and on a green matter basis for the Fairway selections where the clones showed little variation in moisture content of the forage at harvest time. The yield in the clonal nurseries is the number of grams taken from one replication or clonal row which consisted of five plantlets spaced within a 3-foot row. Only one cutting was taken per year in the clonal nurseries containing the forage type plants. The injury from root rot and an apparent natural summer dormancy seemed to prevent any appreciable production of a second crop of hay. A second cutting was obtained at University Farm in the clonal nursery composed of the Fairway type plants. This second cutting was obtained in the Fairway type because it was resistant to root rot and, therefore, did produce an appreciable yield of forage in September in favorable seasons when the period of summer dormancy following the first cutting was relatively short.

In the studies on seed setting five heads per plant were covered with a parchment paper bag for selfing and five heads were left for open-pollination so that a comparison between the amount of seed set under each condition could be made. The parchment paper bags were 4 inches by 8 inches in size and were held in place by fastening them to a bamboo cane at the top by a string through an eyelet in the top of the bag. The bottom of the bag was tied firmly in order to close it and then tied to the bamboo cane. A small wad of cotton had first been placed around these culms to prevent injury and to exclude the possibility of foreign pollen entering the bag.

The number of seeds produced from five heads under conditions of self-pollination and open-pollination were determined from a germination test of the threshed heads. The selfed seedlings were transplanted from the blotters in the

germinator to sterile soil in flats in the greenhouse. These were transplanted later into the individual plant nursery in the field.

A study was made in one year to determine the seed set under conditions of space-isolation in the forage type. The plants were isolated in wheat and corn fields by at least 200 feet in all directions. However, the growing crops of wheat and corn probably did not materially improve the isolation. Six open-pollinated plants and one one-year selfed progeny of six plants were isolated. In seven cases, the six open-pollinated plants were also isolated in pairs to determine the seed set under such conditions.

Injury from root rot organisms was very severe on the forage type of crested wheatgrass, while the Fairway variety was very resistant. It was soon apparent that one of the important problems in the forage type would be the selection of resistant plants. This somewhat changed the original plan of the breeding program. Notes were taken by scoring the plants from 1 (resistant) to 5 (very susceptible). Some plants were killed during the summer months although in the early spring no evidence of root rot was observed except for the dead crown parts from the previous year. The injury was noted first in May. The notes were taken usually two times during the season; once the last of May and again the last of August.

In studies of pollen size and pollen fertility, the heads were collected in the summer, stored in 70% alcohol and the pollen examined in the fall and winter. As nearly as possible, the heads and anthers were collected one to two days before anthesis.

Chromosome numbers were determined entirely from root tip counts. The roots tips were embedded in paraffin and stained with gentian violet.

In determining the beta carotene content leaf samples were taken from the plants immediately after heading in June. From about 10 culms the third leaf was collected in a glass vial, tightly stoppered, and frozen immediately with dry ice. These samples were then stored at  $-15^{\circ}\text{C}$  until January. At this time, the chemical extractions were made. The extraction procedure used was suggested by Dr. Elmer S. Miller of the Department of Botany after his studies of the methods used by himself and others.

In this study, a 1-gram sample was ground in a mortar with sand and acetone and transferred to a Goldfish extractor for 3 hours. These extracted pigments, in acetone, were transferred to petroleum ether by washing the solution with water and drawing off the water and acetone with the separatory funnel. The solution was treated with diacetone and water as described by Hegsted, Porter, and Peterson (6) to remove the plant pigments other than carotene. After this, the petroleum ether was removed by distillation at reduced pressure to almost dryness and the pigments redissolved in diethyl ether. The samples were transferred to tightly stoppered bottles and stored at  $+2^{\circ}\text{C}$  for 5 to 10 days. The concentration of beta carotene was determined spectrophotometrically according to the method described by Miller (10). The spectroscope used was of the type described by Hogness, Zscheile, and Sidwile (7). The results are given in percentage of total dry weight. The method of reading the sample in the photoelectric spectrophotometer and the method of calculation as used in this study are given in detail by Schmid (16). The absorptions were determined at the wave length of  $4.525\text{ }\mu$  which is the maximum absorption for beta carotene as determined by Miller (10).

## EXPERIMENTAL RESULTS

## CHROMOSOME NUMBERS

The chromosome number for 33 open-pollinated plants of the forage type of crested wheatgrass was determined. Of this group, 31 seemed to have a somatic chromosome number of 28; one plant, 58-37, had 29 or 30; and one had 14. The plant with 14 chromosomes was morphologically similar to the Fairway type and was considered to be a Fairway plant. The Fairway plants examined in this study all had a  $2n$  number of 14 chromosomes.

One-year selfed progenies from three plants of the forage type which had 28 chromosomes were studied for chromosome number. These three progenies consisted of a total of 15 plants. All of these plants appeared to have the normal  $2n$  number of 28 chromosomes.

## STUDIES ON SEED SETTING, POLLEN SIZE, AND POLLEN FERTILITY

Both types of crested wheatgrass were highly unfruitful when self-pollinated. Only one plant, 57-31, which was from the forage type, seemed to possess any appreciable amount of self-fertility under the conditions of these tests. Some one-year selfed plants and 58-37, the aneuploid plant from the forage type, failed to produce an appreciable amount of seed under open-pollinated conditions. None of the one-year selfed plants showed an increase in seed setting over their parental plants under conditions of self-pollination. The one-year selfed progeny plants from 57-31 were very unfruitful under conditions of self-pollination and some of them produced no seed from open-pollinated heads when space-isolated. This may have been caused in part by a lack of vigor which was very noticeable in this selfed progeny. All selfed progenies studied in both types of crested wheatgrass tended to be reduced in vigor in comparison to seedlings from open-pollinated seed and to show some chlorophyll abnormalities and dwarfed or twisted leaves. It seemed that no definite genetical ratios could be determined from these data by disomic or tetrasomic inheritance.

The results of seed setting under conditions of space-isolation, open-pollination, and self pollination are given in Table 1 for a few plants of the forage type.

From these data it seems that a definite increase in seed setting may be obtained under conditions of space-isolation as compared to that obtained under parchment bags. Plant number 57-31, which gave a comparatively high amount of selfed seed under bags, seemed to be as fertile under the above conditions of isolation as under open-pollination in the individual plant nursery. If the isolation were complete, the amount of self-unfruitfulness may not be as high as is indicated by artificial selfing as practiced in these and similar studies.

The few comparisons of seed setting obtained when two plants were isolated together are interesting. The plants all flowered at approximately the same time. Indications are that increased fertility is obtained from pollen from another plant in some cases, but not in others. Cross-compatibility may not exist in all cases.

TABLE 1.—Seed setting under conditions of space-isolation as compared with seed setting under self- and open-pollination in the individual plant nursery.

Plant No.	Seedlings from five heads		
	Open-pollinated	Self-pollinated	Space-isolated
58-22 (non-selfed) . . . . .	394	5	78
58-27 (non-selfed) . . . . .	159	1	0
58-40 (non-selfed) . . . . .	84	1	10
58-43 (non-selfed) . . . . .	189	2	5
58-48 (non-selfed) . . . . .	204	9	51
57-31 (non-selfed) . . . . .	205	85	256
57-31-1 (1-year selfed) . . . . .	—	—	1
—6 (1-year selfed) . . . . .	22	1	7
—7 (1-year selfed) . . . . .	11	0	0
—9 (1-year selfed) . . . . .	0	0	9
—12 (1-year selfed) . . . . .	0	0	2
—20 (1-year selfed) . . . . .	110	0	0
58-22 + 58-27 (non-selfed) . . . . .	—	—	250
58-22 + 58-40 (non-selfed) . . . . .	—	—	43
58-22 + 58-48 (non-selfed) . . . . .	—	—	100
58-27 + 58-40 (non-selfed) . . . . .	—	—	12
58-27 + 58-48 (non-selfed) . . . . .	—	—	3
58-40 + 58-43 (non-selfed) . . . . .	—	—	34
58-43 + 58-48 (non-selfed) . . . . .	—	—	110

Measurements were made of pollen size and pollen fertility on some open-pollinated forage and Fairway plants and on some one-year and two-year selfed progenies of the forage type.

The groups of one-year and two-year selfed plants of the forage type produced smaller and fewer normal pollen grains than the open-pollinated plants. However, some one-year and two-year inbred plants produced pollen of the same size and fertility as the open-pollinated plants. It was apparent that selfing tended to increase the variability between plants in pollen size and pollen fertility.

Another point of interest was the lack of difference in pollen size between the tetraploid forage type plants and the diploid Fairway plants.

#### STUDIES OF SELECTED PLANTS IN CLONAL PROGENIES

A summary of the data on the clonal nurseries is presented in Table 2. The first clonal nursery was composed of plants selected from a seed source of the forage type introduced from Canada. The nursery was composed of two replications each at University Farm and Waseca. The second clonal nursery was composed of plants of the forage type selected from the seed source from Montana. The same characters were studied as in the first clonal nursery. This nursery was grown at University Farm only and contained three replications. The third clonal nursery contained the plants selected from the Fairway variety. This nursery was grown at University Farm and Waseca. Two replications were used at each location. These plants from the Fairway variety were resistant to root rot and were rather similar in morphological characters. The only data taken were on the yield

TABLE 2.—Summary of data from clonal nurseries.

Number and kind of plants studied	Individual plant yield, grams dry weight	Clonal yield, grams dry weight	Plant height, inches	Root rot injury score	Number of clones significantly lower in yield than the average of the checks	Number of clones not significantly different in yield than the average of the checks	Number of clones significantly higher in yield than the average of the checks
Range of mean values of yield, plant height, and root rot injury of the clonal progenies of plants of the forage type selected from the seed source from Canada and grown in clonal nurseries at University Farm and Waseca and their yields in the individual plant nursery grown at University Farm							
56, forage type.....	121-469	175-745	22-34	1.9-4.4	37	16	0
4, Fairway type (checks)	—	651-716	22-26	1.1-1.4	—	—	—
Min. level of significance	—	138	1.0	0.6	—	—	—
Range of mean values of yield, plant height, and root rot injury of the clonal progenies of plants of the forage type selected from the seed source from Montana and grown in a clonal nursery at University Farm and their yields in the individual plant nursery grown at University Farm							
29, forage type.....	156-558	342-877	28-41	1.0-4.8	20	8	1
6, Fairway type (checks)	—	578-776	26-30	1.0-1.2	—	—	—
Min. level of significance	—	150	2.3	0.6	—	—	—
Range of mean values of yield of the clonal progenies of plants selected from the Fairway variety and grown in clonal nurseries at University Farm and Waseca							
83, Fairway type.....	—	903-1,671*	—	—	0	39	44
5, forage type (checks)	—	707-1,088*	—	—	—	—	—
Min. level of significance	—	198*	—	—	—	—	—

\*Green weight.



of the clonal progenies. The analysis of variance was used to determine the experimental error and the minimum level of significance at the 5% point determined from  $t \times \text{S.E.}$  of the difference is given for each character studied.

The differences among the clonal progenies from the forage type were highly significant for yield, height, and root rot injury. The differences in yield among the clonal progenies from the Fairway variety were also highly significant.

In general, the Fairway clones tended to be higher yielding than the clones of the forage type. In addition, the Fairway clones were distinctly more resistant to root rot. However, some clones of the forage type were as high yielding as the check clones of the Fairway and approached them in root rot resistance. One clone of the forage type in the second nursery was significantly higher yielding than the Fairway checks and was resistant to root rot. This plant should be of value as a source of material for further breeding studies. In the clonal nursery of the selected Fairway clones, many of the plants tested significantly outyielded the check clones of the forage type. From these facts it appears that both types of crested wheatgrass can be improved by selection of superior plants after they have been tested in replicated clonal nurseries, but that the Fairway type shows greater promise than the forage type under the environmental conditions at University Farm and Waseca.

As stated previously, injury from root rots was very severe in the forage type of crested wheatgrass, while the Fairway variety was nearly free of root rot damage. The injury occurred mainly during the months of July and August after the forage had been removed from the plots. The early spring and late fall growth seemed free of any great injury and was affected only by the root rot and killing which had occurred during the summer of the previous year. However, injury in the form of early maturity or drying was sometimes noted soon after heading. The occurrence of this injury was no doubt responsible in part for the wide variation obtained in yield and morphological characters. It was severe in the seedlings during their first summer and on newly established clonal lines. Many individual plants and parts of clonal rows died. It seemed that the forage type of crested wheatgrass yielded very well during the cooler weather of the spring and fall months but is really unadapted because of the injury from root rots and hot weather during the months of July and August. The Fairway variety shows little injury during the summer months from root rots and did produce enough growth in the fall to permit the taking of a second cutting in some tests, indicating a quicker recovery from the hot weather of the summer months.

The presence of this extreme injury from root rots greatly hindered all studies with the forage type. The injury exerted a marked influence on yield. A correlation coefficient was calculated for yield and the amount of root rot in both clonal nurseries of the forage type. The correlation in both tests of  $r = -.80$  was highly significant and indicated that a high incidence of root rot was associated with low yield. Perhaps the yield data in the forage type were a better measure of root rot resistance than of the true yielding ability.

A point of interest in the clonal line nurseries was the relationship between the yield of these selected plants grown in the individual plant nursery and their yield when tested as clonal progenies. A correlation coefficient of  $r = +.28$  was obtained with plants of the forage type tested in the first clonal nursery and  $r = -.10$  for the plants tested in the second clonal nursery. The first correlation coefficient just reaches the 5% level of significance, whereas the second is not different from zero. This lack of any appreciable relationship was probably due to the great variability in yield of individual plants due to environmental influences. In addition, the effect of root rot may have been partly responsible for reducing any relationship which might have existed.

#### EFFECTS OF SELF-FERTILIZATION ON YIELD AND ROOT ROT INJURY

Fourteen one-year selfed progenies were studied as individual plants for their variation in yield and root rot injury. Each progeny contained four or more plants. Unfortunately, no individual plants from open-pollinated seed sources were suitable for comparison with the one-year selfed plants of the forage type. However, the variation for yield and root rot injury within and between the selfed progenies should be of interest. The data are given in Tables 3 and 4.

TABLE 3.—Frequency distribution of one-year selfed plants of the forage type for yield in grams on a dry matter basis.

Non-selfed parental plant	Individual plant yield, 1937	One-year selfed progeny yield in grams, 1938					Total plants	Progeny mean
		0-100	101-200	201-300	301-400	401-500		
57-10	255	1	2	—	3	1	7	261±133
-20	384	—	2	2	2	1	7	298±112
-30	243	—	3	2	1	—	6	230±95
-31	301	2	3	4	—	1	10	212±147
58-4	75	1	3	1	6	—	11	262±118
-11	308	—	4	6	1	—	11	220±51
-12	290	—	3	—	3	—	6	228±99
-18	225	—	2	2	—	—	4	197±42
-20	393	—	—	3	1	—	4	281±54
-24	303	—	3	3	4	—	10	243±80
-26	397	—	—	1	2	1	4	346±68
-32	304	—	3	2	2	—	7	241±91
-35	305	—	1	4	—	—	5	256±52
-48	325	8	7	3	—	2	20	149±118

A wide variation in yield and root rot injury was found within most of the selfed progenies. There were marked differences between the mean yields of some progenies. None of the progenies showed any great resistance to root rot, although a few individual selfed plants appeared to have some resistance.

In neither of these characters was there any marked relationship between the means of the progenies and the performance of their parental plants. In the case of yield, the effect of root rot, and the small numbers in the progenies may have removed extreme variation

TABLE 4.—*Frequency distribution of one-year selfed plants of the forage type for root-rot injury.*

Non-selfed parental plant	Mean from clonal nursery, 1938-40	One-year selfed progeny score for root rot 1938					Total plants	Progeny mean
		1	2	3	4	5		
57-10	3.5	—	1	4	—	2	7	3.4±1.1
-20	4.0	—	—	3	3	1	7	3.7±0.8
-30	2.4	—	—	1	2	3	6	4.3±0.8
-31	3.1	—	—	2	2	6	10	4.4±1.0
58-4	3.5	—	3	3	3	3	12	3.5±1.2
-11	3.7	—	1	3	3	4	11	3.9±0.9
-12	2.5	—	2	3	—	1	6	3.0±1.1
-18	4.3	1	—	—	1	2	4	3.8±1.9
-20	2.8	—	1	—	—	5	6	4.4±1.2
-24	2.6	—	4	—	—	7	11	3.9±1.5
-26	3.7	—	4	—	—	2	6	2.9±1.4
-32	4.3	—	—	—	2	5	7	4.7±0.5
-35	3.4	—	—	—	1	4	5	4.7±0.4
-48	2.8	—	1	4	6	9	20	4.2±0.9

in individual plant yields or masked any significant association between the parent plants and the progeny means. In the case of the root rot injury the measure of the parental plants was their mean value for root rot damage taken in the replicated clonal nurseries. This might not be expected to show any great relationship to the inbred progeny means because of the great variability in root rot injury of the individual selfed plants due to environmental and soil conditions.

Sufficient numbers of one-year selfed progenies were obtained from five of the original selected Fairway plants so that a study of the variation in yielding ability on the individual plant basis could be made. These results are given in a frequency distribution in Table 5, together with a group of open-pollinated plants for comparison.

TABLE 5.—*Frequency distribution of one-year selfed plants of the Fairway variety for yield in grams on a dry matter basis.*

Non-selfed parental plant	Mean clonal yield, grams green weight	One-year selfed progeny yield in grams dry weight					Total plants	Progeny mean
		1-150	151-300	301-450	451-600	601-750		
51-32.....	1249	2	—	3	1	—	6	304±186
52-24.....	1199	—	1	5	3	—	9	405± 69
52-34.....	1252	—	—	5	4	—	9	433± 98
54-13.....	1167	4	11	3	—	—	18	200± 92
54-17.....	1281	—	—	5	13	1	19	484± 62
Unselected	—	—	1	28	20	1	50	422± *86

A wide variation was observed within and between these selfed progenies. This was similar to the results with the selfed progenies

from the forage type. Two progenies were slightly lower and one distinctly higher in variation in plant yield than the group of open-pollinated plants. No great relationship was observed between the yield of the parental plant and the mean yield of their progenies. A few of the selfed plants yielded materially less than the open-pollinated plants. Some one-year selfed plants yielded as well as the higher yielding open-pollinated plants, but none exceeded them.

One very important point of interest is the fact that one one-year selfed progeny of the Fairway type progeny yielded more, as measured by the means, and two yielded as well as the open-pollinated plants. From these results it seems evident that inbred lines that have been selfed for one year could be selected in the Fairway variety that did not show a reduction in vigor in comparison to unselected open-pollinated plants.

#### STUDIES OF BETA CAROTENE PIGMENT CONTENT

In the determination of beta carotene, duplicate leaf tissue samples were analyzed whenever sufficient material had been collected. This was a technic study designed to determine the error on the method of chemical analysis used in this study. Eighty-four duplicate samples were analyzed and the analysis of variance run on these with the variation determined between plants and within plants.

The percentage of beta carotene was presented on a dry matter basis. The range in the amounts of beta carotene in these samples was from  $1.34 \times 10^{-4}$  to  $3.95 \times 10^{-4}\%$ . The standard error of a single determination was  $.191 \times 10^{-4}\%$  of beta carotene which, given as a percentage error, would be 7.23. This seems to be a satisfactorily low sampling error.

Sixteen plants which were tested in clonal progenies in two replications each at University Farm and Waseca were analyzed for beta carotene content.

The range in means for these clonal progenies was  $2.40 \times 10^{-4}$  to  $3.26 \times 10^{-4}\%$  beta carotene on a dry matter basis. After determining the error from the analysis of variance no significant differences were found among the clones.

#### SUMMARY

The purpose of these investigations was to study methods of breeding crested wheatgrass. The primary objectives were to study the variability among plants tested in individual plant nurseries and as replicated clonal progenies; and to determine the effects of self-fertilization. These studies were made with plants selected from both the forage and Fairway types.

The plants from the forage type had a normal somatic chromosome number of 28 and those from the Fairway type 14. In the forage type only one plant, 58-37, out of 33 had a definite aneuploid chromosome number. Fifteen one-year selfed plants from three different progenies of the forage type all had a  $2n$  chromosome number of 28.

Both types of crested wheatgrass were highly unfruitful when self-pollinated. Only one plant, 57-31, which was from the forage type

seemed to possess any amount of self-fertility under the conditions of these tests. Some one-year selfed plants and 58-37, the aneuploid plant from the forage type, failed to produce any appreciable amount of seed under open-pollinated conditions. No one-year selfed plants showed an increase in seed setting under conditions of self-pollination over that of their parental plants.

The one-year selfed progeny plants from 57-31, the one plant which did possess some degree of self-fertility, were very unfruitful under conditions of self-pollination and some of them produced no seed from open-pollinated heads when spaced-isolated. This may have been caused partly by a lack of vigor which was very noticeable in this selfed progeny.

In the forage type some plants produced more seed under conditions of space-isolation than under parchment paper bags. The somewhat self-fertile forage type plant, 57-31, seemed to be as self-fertile when space-isolated as under conditions of open-pollination in the individual plant nursery. Some plants were cross-compatible and others were not when space-isolated in pairs.

Some of the one-year and two-year selfed plants produced pollen distinctly smaller in size and showed a reduction in pollen fertility when compared with non-selfed plants, while others were not different. The few non-selfed forage and Fairway type plants studied were not different in pollen size or pollen fertility.

Significant differences in yield, plant height, and root rot injury were found among the plants of the forage type which were tested in replicated clonal progenies. The differences in yield among selected plants of the Fairway type tested in replicated clonal progenies were significant. Plants of the Fairway type were significantly higher yielding and shorter in plant height than the forage type and were resistant to root rot injury.

The plants from the forage type showed severe injury from root rots during the months of July and August. This injury greatly affected yield as shown by a highly significant correlation coefficient of  $r = -.80$ .

Most of the one-year selfed plants were greatly reduced in vigor. However, a few one-year selfed plants when tested as clonal progenies seemed to be as vigorous as their parental plants. Some one-year selfed progenies of the Fairway type were as vigorous and one progeny was more vigorous as measured by their mean yield than a group of unselected open-pollinated plants. The variability in yield and root rot injury were rather great within most selfed progenies. The relationships between the character values of the parents and the mean character value of their one-year selfed progenies were very small or lacking.

There were no significant differences among 15 plants of the forage type in beta carotene content when they were tested as clonal progenies in replicated trials at University Farm and Waseca.

From these studies it seems that a further improvement over individual plant selection can be made in crested wheatgrass by selection among these superior plants after they have been tested in replicated clonal progenies. In addition, it seems that the study of

and selection within selfed progenies in crested wheatgrass does not appear to be a very promising means of improvement because of the difficulty of obtaining selfed seed and because of the lack of vigor and uniformity as observed in many of the one-year selfed progenies. However, a few one-year selfed progenies from the Fairway variety were as desirable as unselected open-pollinated plants. If selection within selfed progenies is to be used, it appears that a greater search for lines or strains which set seed under conditions of self-fertilization will be necessary.

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## COMPARISON OF THE EFFECT OF CLIPPING AND GRAZING TREATMENTS ON THE BOTANICAL COMPOSITION OF PERMANENT PASTURE MIXTURES<sup>1</sup>

MASON A. HEIN AND PAUL R. HENSON<sup>2</sup>

IN evaluating various strains of pasture species in small plots, workers have had to resort to clipping to determine the productivity of these species. Even if time and facilities were available to determine the productivity of new or improved species under grazing, seed stocks are usually inadequate for these tests. Hence, it is highly important that the methods employed in testing new strains approach the grazing practices of that particular region. Robinson, Pierre, and Ackerman (6),<sup>3</sup> Gardner, *et al.* (3), and Brown (1) have reported correlated or relatively close agreement between yields of clipped vs. grazed pastures. The literature contains little information as to the comparative effect of clipping and grazing on the botanical composition of permanent pasture mixtures.

The studies reported here compare the effects of clipping and grazing on the botanical composition of various pasture mixtures sown at Beltsville, Md.

### PROCEDURE

Eight mixtures of various grasses in combination with Louisiana white clover in replicated plots were studied under frequent clipping and grazing with sheep. The details of the seedbed preparation and fertilization have been discussed by Henson and Hein (4). Before preparing the seedbed for the mixtures, the area received 2,000 pounds of limestone, 600 pounds of 16% superphosphate, 200 pounds of muriate of potash, and 14 tons of well-rotted manure per acre. The mixtures, as listed in Table 1, were sown in triplicate 5×12 foot plots September 29, 1936. Excellent stands were secured on all plots.

On the mowed plots, the herbage was clipped with a lawn mower to a height of 1¼ inches whenever it had reached a pasture stage of growth (4 to 6 inches). All plots were clipped in 1937, nine clippings being obtained. The first replicate of each mixture was clipped in this manner throughout the 4-year period, 1937-40. The second and third replicates were grazed by sheep during the three grazing seasons 1938-40. Sheep in sufficient numbers to graze the area uniformly in 3 days or less were placed on all the plots when the herbage had reached a 4- to 6-inch height. While the plots were usually uniformly grazed, tall and uneaten herbage was cut with a scythe immediately after grazing. It was not always possible to graze and mow the plots at the same time, therefore, botanical readings were made at times when the stage of growth of the herbage under the two treatments was comparable.

The relative frequency or density of the species in the swards of the various mixtures was determined by making five random counts on each plot with the

<sup>1</sup>Contribution from the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture. This experiment was conducted at the Beltsville Research Center, Beltsville, Md., on land furnished by the Bureau of Dairy Industry. Received for publication February 2, 1942.

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 573.

TABLE 1.—Seed mixtures and rates of seeding of the permanent pasture mixtures.

Species	Rate of seeding in pounds per acre for mixture							
	I	II	III	IV	V	VI	VII	VIII
Kentucky bluegrass...	20	18	10	8	8	8	—	—
Redtop.....	—	3	3	3	—	—	—	—
Orchard grass.....	—	—	8	6	5	5	—	—
Timothy.....	—	—	—	4	4	4	—	—
Italian ryegrass.....	—	—	—	—	4	—	—	—
Perennial ryegrass....	—	—	—	—	—	4	—	—
Canada bluegrass.....	—	—	—	—	—	—	20	—
Colonial bent.....	—	—	—	—	—	—	—	20
Louisiana white clover	4	3	3	3	3	3	4	4
Total.....	24	24	24	24	24	24	24	24

inclined point quadrat. As each needle was pushed to the ground all species coming in contact with the point of the needle were identified and recorded. The relative frequency of a species, according to Levy (5), is the total number of times the species is struck by all needles at all locations within any area. The first reading on all plots was made October 31, 1938. Subsequent readings were made in June and November 1939 and in May and October 1940. The relative frequency of the sown and volunteer species and weeds under clipping and grazing at the different dates is given in Table 2.

## RESULTS

As Kentucky bluegrass has been the dominant species since the initial counts were made, the data are not necessarily indicative of what would have happened to the other grass species under clipping and grazing had Kentucky bluegrass not been included.

The relative frequency of the total grass species in the eight mixtures under clipping and grazing was analyzed for significance by analysis of variance. Eisele and Aikman (2), in Iowa in a study of pasture plants, reported, "That for a given area there is an optimum number of plants of similar requirements that the area can support". Similar results have been obtained in this study. It was found that while there was a significantly higher frequency of grass species under grazing, 149.7 as compared to 133.0 in the clipped plots, the total frequency of the grasses in the various mixtures was not significantly different. Since the clippings were removed and the droppings were allowed to remain on the grazed plots, it is probable that the higher soil fertility level was largely responsible for the greater relative frequency of grasses under grazing.

### BEHAVIOR OF DIFFERENT SPECIES

*Kentucky bluegrass.*—The relative frequency of Kentucky bluegrass was from 7 to 28% higher under grazing than under clipping in all mixtures except No. VI, in which the low frequency of Kentucky bluegrass was undoubtedly due to the much higher relative frequency



TABLE 2.—Relative frequency of plant species under clipping and grazing in the cover of plots sown to various pasture mixtures at Beltsville, Md., 1938-40.

Species	Oct. 31, 1938		June 13, 1939		Nov. 1, 1939		May 23, 1940		Oct. 14, 1940		Average	
	Clipped	Grazed	Clipped	Grazed	Clipped	Grazed	Clipped	Grazed	Clipped	Grazed	Clipped	Grazed
Mixture I												
White clover.....	8	11	10	16	8	12	27	52	2	8	11.0	19.8
Ky. bluegrass.....	103	143	98	80	91	121	187	193	106	107	117.0	128.8
Total weeds.....	20	12	0	1	11	7	1	2	18	4	10.0	5.2
Volunteer:												
Grasses.....	0	0	3	4	6	0	9	5	7	1	5.0	2.0
Legumes*.....	0	0	0	0	0	0	5	1	0	0	1.0	0.2
Mixture II												
White clover.....	0	22	6	15	8	12	17	26	0	6	6.2	16.2
Ky. bluegrass.....	86	103	77	71	109	99	172	179	98	132	108.4	116.8
Redtop.....	22	23	13	25	8	16	19	38	6	12	13.6	24.8
Total weeds.....	17	10	2	0	4	11	1	4	37	3	12.2	5.6
Volunteer:												
Grasses.....	0	0	0	0	0	0	0	0	0	0	0	0
Legumes*.....	0	0	0	1	0	0	4	0	4	0	1.6	0.2
Mixture III												
White clover.....	10	13	8	14	17	8	18	13	3	8	11.2	11.2
Ky. bluegrass.....	79	117	66	73	79	131	145	175	96	101	93.0	119.4
Redtop.....	17	14	9	8	14	4	15	14	2	5	11.4	9.0
Orchard grass.....	12	17	7	11	11	13	19	8	5	15	10.8	12.8
Total weeds.....	19	9	0	1	1	10	0	8	32	21	10.4	9.8
Volunteer:												
Grasses.....	0	0	0	0	0	0	0	1	0	0	0	0.2
Legumes*.....	0	0	0	0	2	0	18	0	1	0	4.2	0

## Mixture IV

White clover.....	8	14	16	18	15	8	31	27	5	2	15.0	13.8
Ky. bluegrass.....	94	85	49	85	95	123	132	164	105	131	95.0	117.6
Redtop.....	21	27	14	20	1	6	18	18	6	9	12.0	16.0
Orchard grass.....	11	11	4	4	14	7	6	7	3	4	7.6	8.2
Timothy.....	5	2	4	0	2	1	15	5	2	0	5.6	1.6
Total weeds.....	10	11	0	5	4	0	0	3	16	10	6.0	5.8
Volunteer:												
Grasses.....	0	0	0	0	0	0	1	0	0	0	0.2	0
Legumes*.....	0	0	0	0	6	0	9	3	0	0	3.0	0.6

## Mixture V

White clover.....	5	4	36	5	7	9	17	10	1	5	13.2	6.6
Ky. bluegrass.....	96	104	78	93	88	125	162	192	106	130	106.0	132.8
Orchard grass.....	9	19	9	13	24	13	19	5	9	4	14.0	10.8
Timothy.....	7	6	3	1	5	0	11	2	2	0	5.6	1.8
Italian ryegrass.....	3	3	0	4	0	0	0	0	0	0	0.6	1.4
Total weeds.....	3	15	0	0	12	3	4	5	27	9	9.2	6.4
Volunteer:												
Grasses.....	3	1	0	1	0	0	0	0	0	0	0.6	0.4
Legumes*.....	0	0	0	0	2	0	0	1	3	0	1.0	0.2

## Mixture VI

White clover.....	7	7	20	13	9	9	43	23	1	8	16.0	12.0
Ky. bluegrass.....	89	88	55	73	84	75	158	133	105	112	98.2	96.2
Orchard grass.....	8	6	3	5	11	4	12	3	2	9	7.2	5.4
Timothy.....	2	0	0	0	4	0	4	2	2	1	2.4	0.6
Perennial ryegrass.....	10	30	8	13	11	42	6	52	9	13	8.8	30.0
Total weeds.....	34	13	0	1	16	3	17	2	26	14	18.6	6.6
Volunteer:												
Grasses.....	4	1	1	2	0	0	0	0	0	0	1.0	0.6
Legumes*.....	0	0	5	0	7	0	10	0	0	0	4.4	0

\*Black medic.

TABLE 2.—*Concluded.*

Species	Oct. 31, 1938		June 13, 1939		Nov. 1, 1939		May 23, 1940		Oct. 14, 1940		Average	
	Clipped	Grazed	Clipped	Grazed	Clipped	Grazed	Clipped	Grazed	Clipped	Grazed	Clipped	Grazed
Mixture VII												
White clover.....	16	29	35	10	19	10	39	40	4	14	22.6	20.6
Canada bluegrass..	36	67	27	21	20	41	13	28	0	6	19.2	32.6
Total weeds.....	33	17	6	1	1	1	15	3	39	18	18.8	8.0
Volunteer:												
Grasses†.....	46	35	47	52	72	74	156	135	119	122	88.0	83.6
Legumes*.....	0	0	0	0	4	0	13	0	0	0	3.4	0
Mixture VIII												
White clover.....	0	32	3	12	5	14	14	30	6	10	5.6	19.6
Colonial bent.....	171	133	77	72	85	120	114	135	69	61	103.2	104.2
Total weeds.....	8	13	1	0	1	8	2	2	11	10	4.6	6.6
Volunteer:												
Grasses†.....	13	7	14	13	15	20	83	39	64	69	37.8	29.6
Legumes*.....	0	0	0	0	10	0	4	0	2	0	3.2	0

\*Black medic.

†Mostly Kentucky bluegrass.

of perennial ryegrass in the grazed as compared to the clipped plots. The average percentage increase in the relative frequency of Kentucky bluegrass under grazing over clipping for all five counts was 10.1, 7.8, 28.4, 23.8, and 21.5%, respectively, for mixtures I to V. The greater frequency of this species under grazing may be attributed in part to the higher fertility level of the grazed plots. The results of these experiments indicate that Kentucky bluegrass should be grazed to measure the pasture value, but the relative productivity of different strains of this species might be satisfactorily evaluated by clipping, assuming all strains are equal in palatability.

*Louisiana white clover.*—The frequency of white clover in these plots fluctuated widely between the spring and the fall readings. It will be noted from Table 2 that the relative frequency of the white clover in June 1939 and May 1940 is high under both clipping and grazing. Though the average relative frequency in the fall is much lower, there is slightly more white clover on the grazed plots. While these results tend to confirm those of Robinson, et al. (6) who reported more white clover under grazing with dairy cattle than under clipping, the failure of these studies to be entirely in accord may be due to the grazing animals used. Stapledon and Jones (7) in Wales have shown that sheep are not only selective in their grazing but prefer clover to grasses. Under the conditions of this experiment it is quite possible that the sheep grazed the clover more closely than cattle would have grazed it.

*Redtop.*—The relative frequency of redtop was greater under grazing in mixtures II and IV. In mixture II the relative frequency of both redtop and Kentucky bluegrass increased under grazing, whereas in mixture IV redtop increased as the timothy rapidly declined under grazing. In mixtures III (mixture II plus orchard grass) the relative frequency of redtop declined under grazing, whereas orchard grass increased slightly but not significantly.

*Orchard grass.*—The relative frequencies of orchard grass under clipping and grazing in mixtures III and IV were not significantly different. In mixture V, the frequency of orchard grass was higher under clipping, because Italian ryegrass, included in this mixture, went out rapidly in the clipped plots thereby providing space for the maintenance of orchard grass. Mixture V contained much more orchard grass than mixture VI in which perennial ryegrass was substituted for Italian ryegrass. In the grazed plots of the latter mixture it is apparent that the competition from Kentucky bluegrass and perennial ryegrass has been responsible for the decline in the relative frequency of orchard grass. The data indicate that orchard grass in mixtures with short and long-lived perennials under close clipping or grazing is unable to take full advantage of the area formerly occupied by the short-lived species when a long-lived, aggressive perennial which spreads by rhizomes, such as Kentucky bluegrass, is present.

*Timothy.*—Although the relative frequency of timothy in all mixtures in which it was included was low in October 1938, the frequency under grazing has been consistently less than under clipping. As timothy is one of the more palatable species, it is quite possible that sheep removed a greater proportion of the leaf blades by selective

grazing than was removed by clipping. The results indicate that the ability of timothy to remain in the sward under close clipping is not necessarily an indication of its value as a pasture plant.

*Italian and perennial ryegrass.*—The occurrence of Italian ryegrass in the sward of mixture V was last noted in June 1939 and only in the grazed plots of this mixture. In mixture VI the average relative frequency of perennial ryegrass for five counts under clipping and grazing was 8.8 and 30.0%, respectively. This much higher frequency of perennial ryegrass under grazing is due largely to the failure of sheep to graze this species properly. Other workers (7) have reported that sheep preferred clover and orchard grass to ryegrass. In this test, it was noted that in the grazed plots in early summer of each year, the poorly grazed areas usually contained a high percentage of perennial ryegrass. In other investigations at Beltsville, cattle have readily grazed plants of perennial ryegrass which had previously been refused by sheep.

*Canada bluegrass.*—The relative frequency of Canada bluegrass declined rapidly, being replaced by Kentucky bluegrass. The decline, however, has been less severe under grazing than under clipping. The average relative frequency under clipping and grazing for the five counts was 19.2 and 32.6%, respectively.

*Colonial bent.*—The frequency of colonial bent was not consistently reduced by either grazing or clipping. At the last count little if any difference was shown in the relative frequency of this species under clipping or grazing. It may be noted, however, that there was consistently more white clover in the grazed plots of colonial bent.

*Weeds.*—The relative frequency of weed species in the swards of the plots sown to the various mixtures was not significantly different according to the spring and early summer counts. The fall readings, however, showed significantly more weeds, primarily crabgrass (*Digitaria sanguinalis*, Scop.) in the clipped plots.

## SUMMARY

The effects of clipping and grazing with sheep on the botanical composition of eight pasture mixtures are compared.

The relative frequency of Kentucky bluegrass, and to a lesser extent redtop and white clover, increased under grazing. The relative frequency of orchard grass remained unchanged under clipping and grazing, except in combination with perennial ryegrass when it declined under grazing.

A significantly greater frequency of timothy occurred in the clipped plots, although the amount remaining in the plots at the end of the 4-year period was small.

The frequency of perennial ryegrass under grazing was much greater than under clipping. The sheep, however, refused to graze the perennial ryegrass as closely as the other species.

The clipped plots in late summer contained more weeds, particularly crabgrass, than the plots grazed by sheep.

In general, the clipping treatment was more severe than grazing as evidenced by the lower total relative frequency of the grasses under

clipping. The added fertility from the droppings under grazing was undoubtedly partly responsible for this difference.

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## NITROGEN LOSSES FROM ALABAMA SOILS IN LYSIMETERS AS INFLUENCED BY VARIOUS SYSTEMS OF GREEN MANURE CROP MANAGEMENT<sup>1</sup>

RANDALL J. JONES<sup>2</sup>

THE use of legumes as green manure crops has increased remarkably during recent years in the southeastern states. Since this is a region of high rainfall and moderate temperature during the winter months, losses of nitrogen by leaching are frequently high during this season. These facts emphasize the need for information concerning the relative value of different legumes as sources of nitrogen and the influence of various methods of handling the legumes on the yield of the following crop.

Some investigators (5)<sup>3</sup> have found that the increases in yields of crops following certain summer legumes were not proportional to the amounts of green matter turned under. Cowpeas and soybeans used as green manure crops did not give a profitable increase in the yield of the corn crop which followed, according to Mooers (6).

Unpublished results obtained by the Alabama Experiment Station show that summer legumes used as green manure crops did not increase the yield of the following crop as much as might be expected from the amount of nitrogen which was added. In a 2-year rotation of cotton and corn at the Sand Mountain Substation, located on Hartsells fine sandy loam, soybeans grown in the corn gave an increase of 471 pounds of seed cotton per acre in the following cotton crop; whereas, 225 pounds of sodium nitrate (36 pounds of N) produced an 820-pound increase in the yield of seed cotton, according to unpublished data from the Alabama Experiment Station. The average annual yield of soybeans amounted to 6,802 pounds (green weight) per acre which was equivalent to about 50 pounds of nitrogen.

The Alabama data also show that at the Brewton Experiment Field, located on Norfolk sandy loam, an average of 9,213 pounds (green weight) of crotalaria, containing about 45 pounds of nitrogen, turned under annually increased the yield of cotton only 282 pounds per acre; whereas, 225 pounds of sodium nitrate increased the yield of seed cotton 628 pounds per acre. These results were based on a 9-year average.

It is evident from these results that some of the nitrogen supplied by the summer legumes was either lost from the soil or remained in a form unavailable to the following crop. Various investigators (2, 3, 8) have reported that large amounts of nitrogen are leached from sandy

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<sup>2</sup>Assistant Soil Chemist. Grateful acknowledgment is made for the planning of the experiment and installation of the lysimeters by the late Dr. J. W. Tidmore, and for the work contributed by Dr. J. R. Taylor and John I. Wear who conducted the experiment the first two years.

<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 585.

soils when summer legumes are turned under as green manure crops. The present experiment was designed to determine the amounts of nitrogen removed from the soil by leaching and cropping when summer legumes, winter legumes, and sodium nitrate are used as sources of nitrogen and to find a system of soil and crop management which would produce the highest crop yields and also conserve nitrogen.

### PROCEDURE

Three major soil types in Alabama, Norfolk sandy loam, Hartsells fine sandy loam, and Decatur clay loam, were used in this investigation. These soils have been described by Davis (1). The nitrogen and organic matter content, base exchange capacity, and pH of the soils are given in Table 1. Profile samples of each soil were taken in layers and placed in lysimeters in the same order as they occurred in the field. The soil from each layer was thoroughly mixed and screened before it was put in place. The soil column in the lysimeter was 30 inches in diameter and 30 inches deep. The summer legumes used in this experiment were soybeans, cowpeas, and *Crotalaria spectabilis*. These legumes (tops only) were harvested on nearby plots and added while still green to the lysimeters.

### FERTILIZER AND CULTURAL TREATMENTS

The soil in all the lysimeter tanks, except the checks, was fertilized at the rate of 400 pounds of superphosphate and 50 pounds of muriate of potash per acre annually in order that these elements would not be a limiting factor in crop growth. Soybeans were added to certain tanks in October of each year at the rate of 10,000 pounds (green weight) per acre which amounted to about 75 pounds of nitrogen. At the same time cowpeas or crotalaria were added to other tanks in sufficient quantities to give the same amount of nitrogen as that supplied by the soybeans.

The summer legumes were handled in the following ways: (a) Placed on top of the soil in the fall and turned under in the spring, (b) turned under in the fall,

TABLE 1.—*Nitrogen and organic matter content, base exchange capacity, and pH of soils used in lysimeters.*

Soil type	Nitrogen content, %	Organic matter content, %	Base exchange capacity, M.E./100 grams	pH
Norfolk sandy loam. ....	0.019	0.54	1.25	4.85
Hartsells fine sandy loam. ....	0.039	1.26	2.75	4.65
Decatur clay loam. ....	0.078	1.23	8.12	5.0

(c) turned in the fall and the lysimeter covered to prevent leaching, (d) stored until spring and then turned, (e) fall turned and oats grown during the winter, and (f) fall turned and vetch grown during the winter. Two other treatments, without summer legumes, included vetch grown during the winter and turned under in the spring and sodium nitrate applied at the rate of 225 pounds per acre to the summer crop.

Sudan grass was grown during the summer as a test crop in order to determine the influence of the various treatments on crop yield. The amount of nitrogen



removed by the sudan grass was determined by means of the Kjeldahl method. All of the mineral fertilizer was applied just before planting sudan grass, except where winter crops were grown, in which case two-thirds of the fertilizer was applied to the winter crop and one-third to the sudan grass. The winter crops of oats and vetch were turned under during the first week in April and the sudan grass was planted about one month later. The soil in all tanks was turned to a depth of 4 or 5 inches semi-annually in April and October. No further cultivation was given the soils.

Nitrate determinations were made on the leachates by the phenoldisulfonic acid method; no other form of nitrogen was detected. The leachates came through perfectly clear from all tanks. For the first three years nitrate determinations were made after each heavy rain. It was found, however, that the nitrate content of stored samples of leachate did not change within a period of 2 months, therefore, during the last year, nitrate determinations were made every 2 months.

The results reported herein cover a period of 4 years, from October, 1936, to October, 1940, for the Decatur clay loam and Norfolk sandy loam. Only a 3-year average is reported for Hartsells fine sandy loam due to the abnormally high concentration of nitrates in the leachates from this soil the first year. This formation of large amounts of nitrates was probably caused by increased activity of micro-organisms due to aeration as a result of mixing the soil while it was being placed in the lysimeters. The reason why the Hartsells soil responded to this treatment differently from the other soils is not known.

## RESULTS

### PERCENTAGE OF ADDED NITROGEN REMOVED BY LEACHING AND CROPPING WHEN VARIOUS CULTURAL METHODS WERE USED IN HANDLING SUMMER LEGUMES

The cropping system and cultural practices followed determined to a large extent the amount of nitrogen that was lost by leaching. The results given in Table 2 show that the amount of nitrogen leached from Decatur clay loam was negligible, but as much as 70% of the added nitrogen was leached from Norfolk sandy loam. This loss was reduced almost one half by turning the legumes in the spring instead of the fall. With one exception, the amount of nitrogen lost by leaching increased as the percentage of nitrogen in the legumes increased, which was in the following order: *Crotalaria*, cowpeas, and soybeans. The percentage of added nitrogen removed by leaching and cropping will be discussed for the different soil types.

*Norfolk sandy loam.*—The amount of nitrogen leached from Norfolk sandy loam was twice as great when the legumes were turned under in the fall as when they were turned under in the spring. It is evident from Table 3 that considerable leaching of nitrogen occurred when the legumes were left on top of the soil during the winter months. This loss was reduced from approximately 38% to 23%, a difference of 15%, by storing the plants until time for spring turning. When oats were grown as a winter cover crop following fall-turned legumes, the leaching of nitrogen was negligible. It has been found by other investigators (5) that when rye was grown following fall-turned cowpeas, the loss of nitrates during the winter was greatly reduced. A comparison of the results obtained from turning under different

TABLE 2.—*Effect of the kind of legume turned under and time of turning on leaching of nitrogen from different soil types.*

Kind of legume added	Percentage of added nitrogen lost by leaching					
	Norfolk SL*		Hartsells FSL*		Decatur CL*	
	Spring turned	Fall turned	Spring turned	Fall turned	Spring turned	Fall turned
Soybeans.....	37.7	69.6	37.6	51.6	6.3	7.4
Cowpeas.....	32.9	66.4	30.2	41.7	6.0	5.5
Crotalaria.....	29.3	59.2	31.3	52.0	5.1	5.0
Average.....	33.3	65.1	33.0	48.4	5.8	6.0

\*SL = Sandy loam; FSL = Fine sandy loam; CL = Clay loam.

legumes shows that nitrogen losses were highest from soybeans and lowest from crotalaria.

The amount of nitrogen recovered by cropping with sudan grass varied inversely with that lost by leaching. The highest recovery of nitrogen added in the form of legumes was obtained when the legumes

TABLE 3.—*Percentage of added nitrogen removed from different soil types by leaching and cropping.*

Cultural treatment	Percentage of added nitrogen removed					
	By leaching			By cropping		
	Norfolk SL	Hart-sells FSL	Decatur CL	Norfolk SL	Hart-sells FSL	Decatur CL
Soybeans						
Fall turned.....	69.6	51.6	7.4	10.8	32.9	51.3
Spring turned.....	37.7	37.6	6.3	16.2	32.0	40.4
Stored, spring turned	22.8	21.0	4.0	24.7	42.9	48.9
Fall turned, oats winter cover crop.....	14.1	8.3	0	14.1	40.0	25.2
Cowpeas						
Fall turned.....	66.4	41.7	5.5	11.7	30.6	52.7
Spring turned.....	32.9	30.2	6.0	19.1	32.6	44.7
Stored, spring turned	22.2	16.2	2.7	25.0	36.8	43.5
Fall turned, oats winter cover crop.....	12.8	4.1	0	13.3	32.9	26.2
Crotalaria						
Fall turned.....	59.2	52.0	5.0	12.0	31.5	48.3
Spring turned.....	29.3	31.3	5.1	17.7	27.9	37.2
Stored, spring turned	21.1	22.1	3.2	20.7	38.4	38.9
Fall turned, oats winter cover crop.....	6.6	4.7	0	17.7	37.1	29.3

were stored until spring. Those treatments which were most effective in preventing nitrogen from leaching produced the highest yields of sudan grass and likewise resulted in the best recovery of added nitrogen (Tables 4 and 5). This relationship was true in all cases except when oats were grown as a cover crop. Even though the oats were turned while still green and immature, the yield of sudan grass following the oats was very low. The high C/N ratio of the oats resulted in slow decomposition and availability of the nitrogen.

*Hartsells fine sandy loam.*—The results given in Table 3 for the Hartsells fine sandy loam show the same general trend as those obtained on Norfolk sandy loam. However, the losses of nitrogen due to leaching were considerably less in the case of the Hartsells soil when the legumes were turned under in the fall.

The percentage recovery of nitrogen by sudan grass was much higher from this soil than from the Norfolk. Almost the same amount of nitrogen was removed by cropping whether the legumes were turned in the fall or spring. Recovery of nitrogen in the tanks where a winter cover crop of oats had been turned under was approximately as high as it was for any other treatment. In most cases, the total percentages of nitrogen removed from the Hartsells soil by both leaching and cropping were appreciably greater than from the Norfolk soil.

*Decatur clay loam.*—The results given in Table 3 for Decatur clay loam are quite different from those obtained on the sandy soils. Regardless of the method of handling the summer legumes, the loss of nitrogen by leaching from Decatur clay loam was negligible.

The recovery of added nitrogen by cropping exceeded 40% for most of the treatments. However, when oats were grown following soybeans and turned under as a green manure crop, only 25% to 30% of the added nitrogen was recovered. Nitrogen deficiency symptoms were very pronounced on sudan grass which followed the oats. It is apparent from the data obtained that crotalaria decomposes more slowly than either soybeans or cowpeas since less nitrogen was recovered by the sudan grass when crotalaria was used as the source of nitrogen. Recovery of nitrogen was higher on this soil when the legumes were turned in the fall rather than the spring due to the slow rate of decomposition of the plant material.

#### EFFECT OF DIFFERENT SOURCES OF NITROGEN AND VARIOUS TREATMENTS ON REMOVAL OF NITROGEN BY LEACHING AND CROPPING

The annual losses of nitrogen from the different soils under various systems of management are reported in Table 4 as pounds per acre. Sudan grass was grown during the summer on all tanks except the ones which were fallowed continuously. A comparison of the results obtained from the first two treatments shows that on all three soil types leaving the soil bare resulted in high losses of nitrogen by leaching even though no nitrogen was added. By growing sudan grass during the summer this loss was reduced to a very small amount even though the soil was left bare during the winter.

On each soil type the loss of nitrogen from the tanks which received additions of 225 pounds of sodium nitrate per acre was practically the

same as when vetch was grown and turned under as the source of nitrogen. This fact is significant since the sodium nitrate was added as a side-dressing to the summer crop and the nitrogen content of the vetch was two or three times greater than the amount added as sodium nitrate.

TABLE 4.—Average annual loss of nitrate nitrogen by leaching and recovery of nitrogen by sudan grass.

Source of nitrogen and treatment	Nitrogen leached, lbs. per acre			Nitrogen recovered by sudan grass, lbs. per acre		
	Norfolk SL	Hart-sells FSL	Decatur CL	Norfolk SL	Hart-sells FSL	Decatur CL
No nitrogen, fallowed*	30.3	52.8	31.5	—	—	—
No nitrogen.....	15.9	14.6	3.4	4.5	13.5	13.9
No fertilizer.....	19.5	21.3	3.5	2.1	12.0	16.8
36 lbs. N as NaNO <sub>3</sub> ...	15.1	16.7	5.0	20.2	30.5	33.7
Vetch, winter cover crop.....	16.2	16.5	3.0	23.4	42.4	67.0
Soybeans						
Fall turned.....	51.0	38.1	5.4	8.0	24.3	37.5
Spring turned.....	27.6	27.8	4.5	11.9	24.2	27.0
Fall turned, tank covered during winter..	2.0	0	0	29.0	39.8	39.5
Fall turned, oats winter cover crop.....	10.7	6.3	0	10.7	30.0	19.0
Fall turned, vetch winter cover crop.....	45.4	39.3	4.9	55.6	58.3	74.1
Stored, spring turned	16.7	15.5	3.0	18.2	31.9	33.7
Cowpeas						
Fall turned.....	48.5	34.2	4.8	8.3	22.6	38.6
Spring turned.....	23.8	22.5	4.4	12.8	21.7	31.4
Crotalaria						
Fall turned.....	43.4	38.5	4.6	8.7	17.5	35.2
Spring turned.....	21.4	23.2	5.0	13.0	15.5	27.1

\*Sudan grass was grown as the summer crop in all other cases.

The amount of nitrogen leached from the sandy soils which received additions of summer legumes was greatly influenced by the date on which these legumes were turned under and by the cropping system followed. It is interesting to note that growing vetch following fall-turned soybeans allowed very heavy losses of nitrogen from the Norfolk and Hartsells soils, but that practically no leaching of nitrates occurred when oats were grown during the same period. The loss of nitrates increased annually when soybeans were turned under and followed by a crop of vetch, due to the accumulation of large amounts of nitrogen. This is in contrast to the small annual loss of nitrates for the same period when vetch was grown without the addition of soybeans (Fig. 1).

The amount of nitrogen removed from the soils by cropping with sudan grass is shown by the data in Table 4 as pounds of nitrogen per acre. The greatest amount of nitrogen was removed by sudan grass when vetch was used as the source of nitrogen. Nitrogen recovery was highest on Decatur clay loam and lowest on Norfolk sandy loam.

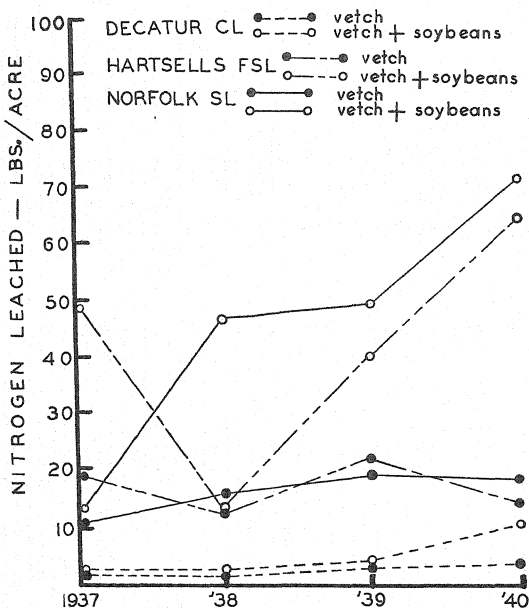


FIG. 1.—Amounts of nitrogen leached from different soils when vetch and vetch plus soybeans were turned under.

#### YIELD OF SUDAN GRASS AS AFFECTED BY DIFFERENT SOURCES OF NITROGEN AND CULTURAL TREATMENTS

The average annual yields of sudan grass for the different treatments on the three soil types are given in Table 5. There were decided differences in the yields resulting from the various treatments which were closely related to the amount of available nitrogen remaining in the soil at the time sudan grass was planted. Yields were much higher on Decatur clay loam than on the sandy soils when the same methods

of fertilization and management were used. The highest yields were obtained on all soils when soybeans were turned in the fall and followed with vetch which was turned in the spring preceding sudan grass.

An average of about 70% more sudan grass was produced on Norfolk sandy loam when the summer legumes were turned in the spring than when they were turned in the fall. Yields of sudan grass on this soil were slightly higher when crotalaria and cowpeas were used as green manure crops than when soybeans were used. The tanks which were covered during the winter to prevent leaching produced higher yields of sudan grass than any other treatment with summer legumes alone. Greater yields were obtained by applying sodium nitrate as a side-dressing than by adding either fall- or spring-turned summer legumes on the sandy soils. When vetch was used as the source of nitrogen, yields of sudan grass were higher than when either summer legumes or sodium nitrate was used.

Sudan grass yields were practically the same on Hartsells fine sandy loam when different summer legumes were added regardless of the

TABLE 5.—Effect of different treatments on the average annual yield of sudan grass and the net gain or loss of nitrogen by different soil types.

Source of nitrogen and treatment	Net gain or loss of nitrogen, lbs. per acre*			Yield of sudan grass, lbs. per acre (dry weight)		
	Norfolk SL	Hart-sells FSL	Decatur CL	Norfolk SL	Hart-sells FSL	Decatur CL
No nitrogen, fallowed	-30.3	-52.8	-31.5	—	—	—
No nitrogen . . . . .	-18.5	-26.4	-14.8	224	1,046	1,770
No fertilizer . . . . .	-19.9	-31.6	-18.6	523	1,382	1,687
36 lbs. N as NaNO <sub>3</sub> . . .	3.0	-8.6	3.0	2,142	3,169	3,912
Vetch, winter cover crop . . . . .	-9.5	66.4	51.7	2,010	3,534	5,613
Soybeans						
Fall turned . . . . .	14.3	11.5	30.3	836	2,625	3,721
Spring turned . . . . .	33.7	22.0	36.7	1,452	2,563	3,477
Fall turned, tank covered during winter . .	42.4	33.6	33.7	3,144	4,250	4,222
Fall turned, oats, winter cover crop . . . . .	53.7	38.7	56.0	1,050	3,424	1,809
Fall turned, vetch, winter cover crop . . .	42.9	65.7	103.9	4,137	4,799	6,298
Stored, spring turned	38.3	26.6	36.6	2,274	3,651	3,941
Cowpeas						
Fall turned . . . . .	14.2	14.4	27.6	939	2,631	4,149
Spring turned . . . . .	34.4	27.1	35.2	1,676	2,670	3,960
Crotalaria						
Fall turned . . . . .	21.1	12.2	34.4	944	2,712	3,681
Spring turned . . . . .	38.8	30.1	42.4	1,511	2,060	3,398

\*Gains and losses of nitrogen represent calculated differences rather than results by analysis of soil.

time of turning, except for spring-turned *crotalaria* which gave a lower yield.

On Decatur clay loam, higher yields were obtained from fall-turned than from spring-turned summer legumes in every case. Yields of sudan grass following vetch far exceeded those obtained from turning summer legumes on this soil.

#### INFLUENCE OF VARIOUS SYSTEMS OF MANAGEMENT ON NITROGEN BALANCE OF DIFFERENT SOILS

The data presented in Table 5 reveal that extensive losses of nitrogen occurred when the soils to which no nitrogen had been added were fallowed continuously. The annual loss of nitrogen by the fallowed soils may be observed in Fig. 2. The sandy soils showed an annual decrease after the first year, but the loss of nitrogen by leaching increased annually on Decatur clay loam. The nitrogen level of all soil types remained practically unchanged when sodium nitrate was used as the source of nitrogen. However, growing vetch on the Hartsells and Decatur soils resulted in a net annual gain of 66 pounds and 52 pounds of nitrogen per acre, respectively. When vetch was grown following fall-turned soybeans, the annual net gain in nitrogen amounted to 43 pounds on Norfolk, 66 pounds on Hartsells, and 104 pounds on Decatur.

The annual gain in nitrogen when the legumes were spring turned was twice as great as when the legumes were turned in the fall on the Norfolk and Hartsells soils. The addition of *crotalaria* resulted in slightly higher gains in nitrogen than were obtained when soybeans or cowpeas were used.

Obviously, where good yields of vetch can be produced, growing this crop is the best method for increasing the nitrogen content of soils. If summer legumes are used on sandy soils, twice as much nitrogen will be retained if they are turned in the spring rather than in the fall. Vetch plus fall-turned soybeans produced the highest gains in soil nitrogen, but this resulted in an accumulation of large amounts of nitrates which were not used economically on the sandy soils because of leaching.

#### AMOUNT OF RAINFALL PASSING THROUGH LYSIMETERS

The rainfall during the period of the experiment varied from 45 to 55 inches annually. The percentage of the rainfall which leached through the Decatur clay loam and Hartsells fine sandy loam was practically the same and varied from 35% to 40%, depending on the treatment used. The Hartsells soil seemed to pack and become somewhat impervious in the lysimeters which accounts for the low rate of percolation. It is probable that more water would leach through this soil under field conditions. From 55% to 65% of the rainfall was obtained as leachate from the Norfolk sandy loam. The remaining water was lost by evaporation from the soil and transpiration from the plants growing in the tanks.

For each soil type, the amount of water which percolated through the lysimeters was closely related to the crop growth. The lysimeters

which produced the largest crop yields also had the lowest percolation of water. Winter crops further decreased the amount of percolation.

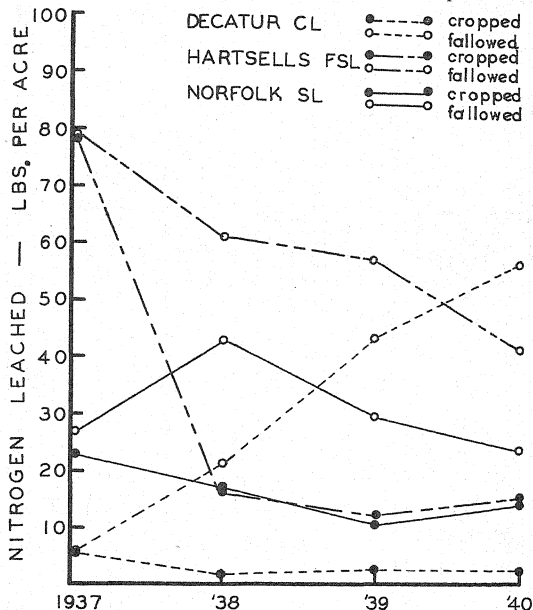


FIG. 2.—Amounts of nitrogen leached from cropped and fallowed soils when no nitrogen was added and sudan grass was the crop grown.

#### SUMMARY AND CONCLUSIONS

The amounts of nitrate nitrogen leached from Norfolk sandy loam, Hartsells fine sandy loam, and Decatur clay loam under different systems of soil and crop management were determined over a 4-year period by the use of lysimeters. Summer legumes, winter legumes, and sodium nitrate were used as sources of nitrogen. Sudan grass was grown during the following summer as a test crop in order to determine the influence of the various treatments on possible crop yields and nitrogen recovery.

The loss of nitrogen by leaching was closely related to the texture of the soil. The amount of nitrogen lost decreased as the clay content



of the soil increased. Only negligible amounts of added nitrogen leached from Decatur clay loam when the soil was cropped, but when this soil was left fallow without the addition of nitrogen, there was a progressive annual increase in the leaching of nitrates which amounted to 56 pounds of nitrogen in 1940. This clearly shows that when nitrates are present they can be readily leached from a clay soil as well as from a sandy soil. Differences in the rate of decomposition of organic material in the soils studied probably account for the higher nitrate losses in sandy soils than in the clay soil.

When summer legumes were added to the sandy soils, nitrogen losses were affected by the kind of legume and time of turning under the legume. The amount of nitrogen leached increased as the percentage of nitrogen contained in the different legumes increased which was in the following order: *Crotalaria*, cowpeas, and soybeans. When soybeans were turned under in the fall, as an average, 70% of the added nitrogen was leached from Norfolk sandy loam and 52% from Hartsells fine sandy loam. This loss was reduced to 38% on both soils when the soybeans were not turned under until spring.

With the soil types studied, the growing of a winter cover crop following fall-turned legumes prevented all but small amounts of nitrogen from leaching. Oats were more effective than vetch in preventing this loss, but the yields of sudan grass were much greater following vetch. When soybeans were turned in the fall and followed with a crop of vetch, which was turned in the spring, the loss of nitrogen increased annually due to the large accumulation of nitrogen and formation of excessive amounts of nitrates.

For the same fertilizer and cultural treatments, the yield of sudan grass on the different soils increased in the following order: Norfolk sandy loam, Hartsells fine sandy loam, and Decatur clay loam. For any one soil type, the yield of sudan grass was directly related to the amount of added nitrogen that was not leached. Higher yields were obtained on sandy soils by applying 36 pounds of nitrogen as sodium nitrate than from 75 pounds of nitrogen in the form of summer legumes. Vetch, grown during the winter and turned under in the spring, produced higher yields of sudan grass than either summer legumes or sodium nitrate.

The nitrogen content of the different soils was maintained at a constant level for 4 years when sodium nitrate was used as the source of nitrogen. The net gain in nitrogen was highest when vetch was grown as the source of nitrogen. There was a net gain in nitrogen by the soils when summer legumes were turned under and this varied inversely with the loss of nitrogen by leaching.

From 55% to 60% of the rainfall percolated through Norfolk sandy loam, and from 35% to 45% through the Hartsells and Decatur soils. It is evident from this that the small loss of nitrate from Decatur clay loam was not caused by a lack of percolation.

From these results it is apparent that summer legumes should be turned in the spring on sandy soils, unless a winter cover crop is to be grown; that winter cover crops greatly reduce the loss of nitrogen by leaching; that the loss of nitrates is related to the texture of the soil; and that soils should not be left fallow during seasons of heavy rainfall.

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## COMPARISON OF DRY COMBUSTION AND WALKLEY-BLACK METHODS FOR THE DETERMINATION OF ORGANIC CARBON DISTRIBUTION IN SOIL PROFILES<sup>1</sup>

CURTIS E. HUTTON AND ROY W. SIMONSON<sup>2</sup>

THE amount and distribution of organic carbon, reflected in the color and thickness of the upper horizons, are distinctive features of the profiles of Prairie soils. Moreover, these properties lend themselves to quantitative measurement and therefore can be useful in the description and classification of Prairie and associated soil groups.

The amounts of carbon in soil samples have been estimated by a number of different methods, among which that of dry combustion is accepted generally as standard (1, 6, 7).<sup>3</sup> Three different methods have been compared by Alexander and Byers (1) and four have been studied by Smith and Weldon (9). The reader interested in detailed comparisons of a number of procedures is referred to the above papers.

The distribution of carbon in soil profiles has been studied in this laboratory by determinations made by the dry combustion method described by Winters and Smith (11). Efforts have been made, however, to find a more rapid procedure that would provide comparable data. Special attention has been given to the method of Walkley and Black (10), since it is rapid and has been used widely (2, 6, 9).

Comparisons of the Walkley-Black method with both dry combustion and wet combustion methods have been reported previously (2, 6, 9), but these studies have not been concerned with carbon distribution in soil profiles. Browning (6) compared data obtained by the Walkley-Black and dry combustion procedures and found that the average recovery of carbon by the former method was 85%. Considerable deviations in recovery from the average were noted with some of the soil samples, however, and it is pointed out that values obtained are approximate.

Baker (2) analyzed samples of the surface horizons of seven soils by the Walkley-Black and by A.O.A.C. wet combustion methods. Recoveries of carbon by the former method ranged from 70.0 to 83.7%, with an average value of 77.2%.

Smith and Weldon (9) studied the hydrogen peroxide, wet combustion, Schollenberger, and Walkley-Black methods. Their study included analyses of a number of samples of surface horizons, analyses of principal horizons in eight profiles and analyses of samples of the surface layer of Marshall silty clay loam from plots on which organic matter restoration experiments had been conducted. The average recovery of carbon in the various groups of samples by the Walkley-Black method, based on amounts determined by wet combustion, ranged from 72 to 76%. Variations in recovery of carbon in the hori-

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<sup>3</sup>Figures in parenthesis refer to "Literature Cited", p. 592.

zons of the eight profiles ranged from 60.5 to 84%, when horizons containing less than 0.5% organic matter are excluded. Walkley and Black (10) in their original work with a group of 20 soils in England reported an average recovery of 76% of the carbon, as determined by dry combustion.

In all of the studies reported, only part of the carbon is oxidized by the Walkley-Black procedure, and this part, as expressed in average figures, ranges from 72 to 85% of the quantity determined by wet or dry combustion methods. Deviations in recovery of carbon for individual samples exceeds the range of the average values.

In the present investigation, determinations by the method of Walkley and Black (10) of organic carbon distribution in the principal horizons of 12 soil profiles were made for comparison with data previously obtained by a dry combustion method (11). The 12 soils are described briefly, and the results obtained are presented, partly in graphic and partly in tabular form. The variations in recovery of carbon in the individual profiles and from one profile to another are discussed, especially as to the effect of the deviations on the usefulness of the Walkley-Black method for the study of carbon distribution in soil profiles.

#### DESCRIPTION OF SOILS

The 12 soils that were studied represent four of the Zonal and two of the Intrazonal groups.<sup>4</sup> The Zonal soils are the Gray-Brown Podzolic, Podzol, Prairie, and Red Podzolic groups, whereas the Intrazonal soils include the Planosol and Wiesenboden groups. Three profiles are of the Prairie group, three are Planosols, two are Wiesenboden, two are Gray-Brown Podzolic or closely related types, one is a Podzol and one is a Red Podzolic soil.<sup>5</sup> The names of the individual soil types, file numbers, Great Soil Groups, and source locations are given in Table 1.

TABLE 1.—*Soils used in the study.*

Soil type	File No.	Great soil group	Source location
Marshall silt loam. ....	P51	Prairie	Iowa
Tama silt loam. ....	P27	Prairie	Iowa
Carrington silt loam. ...	P52	Prairie	Iowa
Webster silty clay loam.	P50	Wiesenboden (meadow soil)	Iowa
Garwin silty clay loam. .	P34	Wiesenboden (meadow soil)	Iowa
Muscatine silt loam. ...	P30	Planosol (grassland)	Iowa
Grundy silt loam. ....	P1	Planosol (grassland)	Iowa
Edina silt loam. ....	P2	Planosol (grassland)	Iowa
Fayette silt loam. ....	P32	Gray-Brown Podzolic	Iowa
Weller silt loam. ....	P4	Gray-Brown Podzolic-Planosol	Iowa
Brassua sandy loam* . . .	P200	Podzol	New Hampshire
Cecil sandy clay loam*	P201	Red Podzolic	North Carolina

\*Descriptions of these soils and data for organic carbon by dry combustion are given by Brown and Byers (4).

<sup>4</sup>The nomenclature followed is that outlined by Baldwin, Kellogg, and Thorp (3).

<sup>5</sup>The samples of Podzol and Red Podzolic soils were obtained through the courtesy of the Division of Soil Survey, Bureau of Plant Industry, U. S. Dept. of Agriculture.

Among the 10 soils from Iowa, only 2, Fayette silt loam and Weller silt loam, were formed under deciduous forest cover and 8 profiles were developed under prairie vegetation. The two soils from forested areas are represented by virgin profiles, whereas the samples of the Prairie and associated soils were collected in cultivated fields. One soil, Webster silty clay loam, has been formed from highly calcareous till of the Late Wisconsin glaciation and another, Carrington silt loam, has been formed where there is a shallow mantle of loess over Iowan till. The other eight soils from Iowa were formed from loess of intermediate texture. None of the samples included in this study contained carbonates, though the deeper horizons of several profiles, notably the Webster, are highly calcareous. The soils have all been described in greater detail in earlier publications (5, 8).

Two soils from regions distinctly different from Iowa were included in the investigation for purposes of comparison. These two soils are Brassua sandy loam, a Podzol formed from glacial till in New Hampshire, and Cecil sandy clay loam, a Red Podzolic soil formed from crystalline rocks in North Carolina. Descriptions and detailed studies of these soils are given by Brown and Byers (4).

## RESULTS AND DISCUSSION

In the classification of the Prairie and associated soil groups, the distribution of carbon within the profile, the total quantity present, and the amounts in the individual horizons are important characteristics. All of these data should be made available in a reliable form by determinations of organic carbon contents in the principal horizons of the profile. For that reason, the recoveries of carbon by the Walkley-Black method, based on the quantities determined by a dry combustion method, are considered in the subsequent paragraphs from three points of view. Attention is given to the deviations in recovery within profiles, to the variations between comparable horizons in different profiles, and to deviations in the average percentage recovery from one profile to another.

The amounts of carbon recovered by the Walkley-Black method are expressed in Fig. 1 as percentages of the contents determined by dry combustion. The graphs show percentage recovery versus depth (in the profile), with all measurements of depth made from the top of the A<sub>1</sub> horizon. The A, B, and C horizons in each profile are shown by squares, triangles, and circles, respectively. Samples of the A<sub>0</sub> horizon (5-0 inches) of Brassua sandy loam were studied, and the recovery in that layer is shown by a point above the body of the graph. All points on the graphs are placed as though they occurred at the center of the layer represented by the soil sample. Broken lines are used to indicate contents of carbon that are less than 0.22% of the soil material. At such low concentrations of carbon, experimental errors become much greater percentagely and the deviations in recovery have less significance.

The curves shown in Fig. 1, excluding that part of each curve shown as a broken line, indicate considerable variations in percentage recovery of carbon from one horizon to another in many of the profiles. The maximum ranges in recovery of carbon within individual profiles are 53 to 79% in Edina silt loam, 53 to 92% in Cecil sandy clay loam, 60 to 80% in Fayette silt loam, and 50 to 74% in Brassua sandy loam.

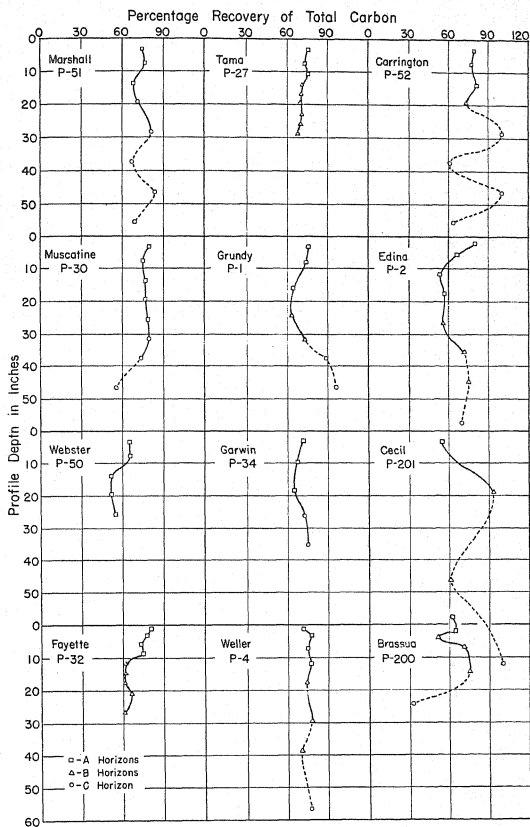


FIG. 1.—Recovery by Walkley-Black method of carbon as determined by dry combustion in principal horizons of 12 soil profiles.

These variations in recovery of carbon from one horizon to another in the same profile are larger than those reported by Smith and Weldon

(9), but they are of the same order of magnitude as the maximum variations between different samples as found by Browning (6). The smallest ranges in recovery of carbon within single profiles are 71 to 78% in Weller silt loam, 72 to 79% in Muscatine silt loam, and 68 to 76% in Tama silt loam. In general, the variations in the recovery of carbon within profiles are smaller in the soils of the Prairie group, though there are exceptions to this tendency.

Comparison of recoveries of carbon in the same horizon of different profiles shows that the differences in percentage oxidized by the Walkley-Black procedure range from as little as 10% to as much as 30%. The smallest variations occur when the comparison is restricted to the same horizon in different profiles of one Great Soil Group, e.g., the Prairie soils. The larger variations exist in recoveries of carbon from the same horizon of soils that differ materially in profile characteristics, as for example, the A<sub>1</sub> horizons of the Webster and Fayette soils. Variations in recovery of carbon by the Walkley-Black method as large as that which occurs between the A<sub>1</sub> horizons of the Fayette and Webster soils (80.2 to 51.4%) obscures one of the important differences between those two soils: A similar difficulty would arise if comparisons were to be made between the Prairie and either the Red Podzolic soil or Podzol profiles. However, as has been pointed out, comparisons can be made of contents of organic carbon in the same horizon of profiles representing one Great Soil Group.

The average percentage recovery of carbon by the Walkley-Black method was calculated for all horizons in each of the 12 profiles studied, and these data are presented in Table 2. The lowest value observed is 57.1% in the profile of Webster silty clay loam, whereas the highest one is 79.2% for the profile of Carrington silt loam. The range in average percentage recovery for the entire profile is lowest among the group of Prairie soils, with figures of 71.7% for Tama silt loam, 74.1% for Marshall silt loam, and 79.2% for Carrington silt loam. The largest difference in average percentage recovery between two closely related soils is that of 13.5% between Fayette silt loam

TABLE 2.—Average percentage recovery of carbon by Walkley-Black method for the entire profile of each of 12 soils.

Soil type	File No.	Percentage recovery of carbon
Marshall silt loam.....	P51	74.1
Tama silt loam.....	P27	71.7
Carrington silt loam.....	P52	79.2
Webster silty clay loam.....	P50	57.1
Garwin silty clay loam.....	P34	69.6
Muscatine silt loam.....	P30	74.0
Grundy silt loam.....	P1	76.0
Edina silt loam.....	P2	65.2
Fayette silt loam.....	P32	61.1
Weller silt loam.....	P4	74.6
Brassua sandy loam.....	P200	58.5
Cecil sandy clay loam.....	P201	76.4
Average recovery for all samples in 12 soils...		71.0

and Weller silt loam. The data given in Table 2, though the figures are averages for different horizons within profiles and thus may not be strictly comparable, further illustrate the range in percentage recovery.

The variations in recovery of carbon by the Walkley-Black method in the separate horizons of a profile or in the comparable horizons of different profiles have been emphasized throughout this discussion. Attention has been given to the deviations rather than to the similarities in results obtained by the Walkley-Black and dry combustion procedures. Earlier papers by Browning (6) and by Smith and Weldon (9) have reported favorable comparisons of the Walkley-Black method with those of dry and wet combustion, and it seems unnecessary therefore to consider the degree of correlation that can be obtained. Browning (6) concludes that the data obtained by the rapid dichromate method are approximate but do have value for some types of studies. Smith and Weldon (9) indicate a need for caution in the interpretation of data on readily oxidizable carbon in terms of carbon contents in their suggestion that the results of the rapid methods be expressed in terms of M.E. of oxidized material per gram of soil. Such data would then be a supplement rather than a substitute for carbon contents obtained by other methods.

The observations made in this investigation are in general agreement with previous findings that there is a high degree of correlation between the data obtained by the Walkley-Black and dry combustion methods. However, the deviations between results obtained by the two methods of analysis are often large, and these deviations frequently obscure important differences in carbon contents within profiles or in similar horizons of widely different soils. On the basis of the results from the study of 12 profiles, the more rapid method of Walkley and Black cannot be considered a satisfactory substitute for a dry combustion method in the study of carbon distribution in soil profiles.

#### SUMMARY

The recoveries of carbon by the Walkley-Black method are compared with data previously obtained by a dry combustion method for the principal horizons of 12 soils representing six Great Soil Groups.

Variations in recovery of carbon range from 53 to 79% in the separate horizons of some profiles, and they reach the same order of magnitude when the same horizons in widely different soils are compared. The smallest deviations occur when data for one horizon in a number of profiles belonging to a single Great Soil Group are compared. It would appear, therefore, that approximate values for organic carbon can be obtained by means of the Walkley-Black method, but the variations in recovery are often large enough to obscure important differences between the various horizons in the same profile or similar horizons in different profiles.

The deviations in recovery of carbon are considered too large to permit the general use of the more rapid procedure for the study of carbon distribution in soil profiles.



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## AGRONOMIC AFFAIRS

### PLANS FOR THE 1942 MEETING OF THE AMERICAN SOCIETY OF AGRONOMY AND THE SOIL SCIENCE SOCIETY OF AMERICA

AS a result of the cancelling of many of the summer meetings of Sections of the Society, there has been some confusion regarding the annual meeting of the American Society of Agronomy and of the Soil Science Society of America. In order to get some measure of the sentiment in favor of cancelling these meetings, the members serving on the committees of both Societies were asked to report to the Executive Committee their views on the two following questions:

1. Should the meetings be held during the present emergency?
2. If so, what topics should receive special emphasis on the program of the annual meeting?

Approximately 90% of those replying felt that the annual meeting was even more important this year than ever. About the same number thought that agronomic problems arising as a result of the war should receive special attention. Many urged less attention to reports of minor investigations and more emphasis upon round-table discussions of emergency problems.

In view of this sentiment of a large representative sample of the membership, the Executive Committee voted unanimously to proceed with plans for the annual meeting. They also recommend to those in charge of organizing programs that special attention be given to emergency problems in accordance with the wishes of the members.

Local arrangements for the meeting to be held in St. Louis, Missouri, November 11 to 13, 1942, are being handled by a committee headed by Doctor W. A. Albrecht of the University of Missouri. The headquarters will be in the glass enclosed, convention section on the 16th floor of the Statler Hotel, between Washington and St. Charles on Ninth Street. This is the downtown hotel section. Special student rates will be available.

### PROGRAM FOR THE CROPS SECTION OF THE AMERICAN SOCIETY OF AGRONOMY

PRELIMINARY plans for the meeting of the Crops Section of the American Society of Agronomy are announced by the Chairman, K. S. Quisenberry, Department of Agronomy, Agricultural Experiment Station, Lincoln, Nebraska. Several sessions will be arranged for contributed papers. Brief papers presenting the results of current research, especially if the results have a bearing on the war effort, are most desirable and are solicited. Those wishing to present papers are requested to send the title to the Chairman of the Section before August 1. An abstract of the proposed paper of not over 200 words must be submitted either with the title, or before August 15.

Other sessions of the Crops Section now planned include a symposium on crops teaching, Dr. L. F. Graber, Chairman; a symposium

on barley breeding and improvement, Dr. D. W. Robertson, Chairman; a symposium on statistics and experimental designs, Dr. H. M. Tysdal, Chairman; and a symposium on soybean breeding, Dr. C. M. Woodworth, Chairman. Several other sessions, including one or two joint programs with the soils men are under consideration.

A general program for the Crops Section will be held during one-half day. At this time topics of wide interest will be discussed and the business of the Section transacted.

#### BIBLIOGRAPHY ON MINOR ELEMENTS

THE Chilean Nitrate Educational Bureau announces publication of the third supplement to the third edition of the "Bibliography of References to the Literature on the Minor Elements and Their Relation to Plant and Animal Nutrition."

The first edition of this Bibliography was published in August 1935, the second edition in November 1936, and the third edition, the last complete edition, in February 1939. Subsequently, the first supplement was published in April 1940, and the second supplement in April 1941.

The latest publication in this series, the third supplement, contains 80 pages and 575 abstracts, which include 106 crops and 32 elements. A total of 691 authors is listed. Complete indices are provided, including an element index, a botanical index, and an author index. In response to requests, the author index has been extended to include the names of all authors listed in the various abstracts. Heretofore only the names of the first author has been shown.

Inquiries regarding the third supplement should be addressed to the Chilean Nitrate Educational Bureau, Inc., 120 Broadway, New York City.

#### BIBLIOGRAPHY ON LITERATURE ON ANALYSES OF LEAF AND OTHER PLANT TISSUES

THIS publication, as the title denotes, is a bibliography of published literature on the analysis of plant tissue material, with special reference to leaf analysis and content of mineral nutrients. The literature covers the period from 1935 through 1940.

Subject matter is arranged by state or country and sub-arranged by author. The index consists of a crop, subject, and author index. The title index is omitted due to its length, and the location index also is omitted because of the geographic arrangement of the bibliography.

The abbreviations of publications are the same as those used by the U. S. Dept. of Agriculture in the *Experiment Station Record*, and the American Chemical Society in *Chemical Abstracts*. Abstracts from both of the above publications have been freely used.

The compilation is the work of Catherine M. Schmidt, Librarian, and Dorothy H. Jameson, Assistant Librarian, of the American Potash Institute, Inc., 1155 Sixteenth Street, N. W., Washington, D. C.

### RECENT PUBLICATIONS OF THE ASSOCIATION OF OFFICIAL SEED ANALYSTS

Three recent publications of the Association of Official Seed Analysts include the 1941 Proceedings of the Association of Seed Analysts, Index of News Letters of the Association of Official Seed Analysts, and History of the Association of Official Seed Analysts. The publications were edited by M. T. Munn of the New York State Agricultural Experiment Station at Geneva.

The 1941 Proceedings of the Association is a 106-page publication which reports the thirty-third annual meeting of the organization. It also contains summaries of reports by the several research committees. These reports deal with such pertinent subjects as methods of germination; identification of weed seeds; and improved laboratory equipment for purity analyses. The contents of the publication should be of value to seed analysts as well as commercial seed dealers.

The 32-page Index of the News Letter begins with volume 1, published in 1927, and includes all copies up to 1941. The Index was prepared by W. O. Whitcomb, Superintendent of the Montana Grain Laboratory, Bozeman, Mont.

The History of the Association of Official Seed Analysts will be of value to all those who are interested in agricultural seed. It contains a historical sketch of individual state laboratories and a list of the outstanding seed analysts in the United States and Canada.

The above mentioned publications may be obtained from Elva L. Norris, Secretary-Treasurer, Association of Official Seed Analysts, Kansas State College, Manhattan, Kans. The 1941 Proceedings, as well as earlier numbers, are \$1.00 per copy. Copies of the Index and History are 75 cents each.

### NEWS ITEMS

THE MAY issue of the *Journal of Geology* contains the five papers on clay delivered at the University of Chicago Fiftieth Anniversary Symposium last September and may be obtained from the University of Chicago Press for \$1.00. The titles of the papers are as follows: Modern Concepts of Clay Materials, Ralph E. Grim; Relation of the Lattice Structure of Clay Minerals to Some Properties of Clays, Sterling B. Hendricks; Applications of Modern Clay Researches in Agriculture, Walter P. Kelley; Applications of Modern Clay Researches in Ceramics, F. H. Norton; and Applications of Modern Clay Researches in Construction Engineering, Hans F. Winterkorn.

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THE ANNUAL meeting of the Canadian Seed Growers' Association was held in Olds, Alberta, June 16 and 17. The headquarters of the Association is located at 251 Besserer Street, Ottawa, Canada.

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AN EXTENSIVE list of institutions, societies, and research workers in the pure and applied plant sciences in Central and South America has been prepared by the editors of *Chronica Botanica* in cooperation



with the Division of Agriculture of the Office of the Coordinator of Inter-American Affairs, Washington, D. C. It has been published in *Chronica Botanica*, Vol. 7, Nos. 2 and 3 (March and May) 1942.

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JOHN H. LONNQUIST, who will receive his Master's degree from Kansas State College this month, has accepted a graduate assistantship in agronomy at the Ohio Agricultural Experiment Station.

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DOCTOR ROBERT M. SALTER, formerly Chief of the Division of Soil and Fertilizer Investigations of the U. S. Dept. of Agriculture, has been named Chief of the Bureau of Plant Industry succeeding Dr. E. C. Auchter who was recently appointed administrator of the Agricultural Research Administration.

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DOCTOR FRED V. GRAU, Pennsylvania State College, has been granted leave of absence from his duties as Extension Agronomist to assist the War Department in turfing air fields. He will act as Associate Chief of the Turf Unit, Construction Branch of the United States Army Engineers, assuming his duties immediately. The position will be that of a civilian attached to the Army, and the work will take him wherever the Army may operate.